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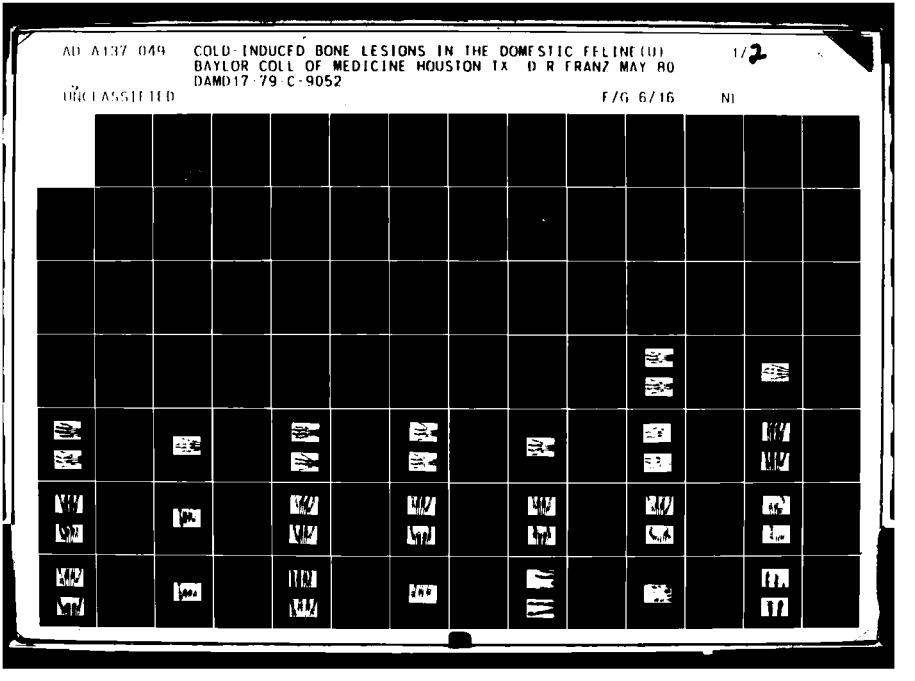

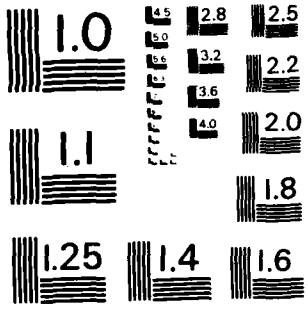

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COLD-INDUCED BONE LESIONS IN THE DOMESTIC FELINE

Final Comprehensive Report

David R. Franz

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ABSTRACT

A limited number of references are made in the literature to skeletal changes following exposure of the extremities to low temperature. The pathophysiology of these lesions has not been described. The lesions have not been reproduced successfully in animals.

One rear foot of each of 30 anesthetized cats was exposed to -50° C moving air to produce a frostbite injury. The animals were studied radiographically, biochemically, and histologically for up to six months following injury. Radiographic changes occurred in 24 animals. Lesions included diffuse or localized radiolucency (22 animals), severe lysis of the bone (four animals), juxta-articular focal loss of density (two animals), periosteal new bone growth (two animals), and early epiphyseal closure (three out of ten animals). Radiographs of injured metatarsals were examined by digital densitometry; all of the feet showed decreased radiodensity at two to six months post-injury. Serum calcium and inorganic phosphorus remained normal throughout the study, although alkaline phosphatase was elevated from two to four months post-injury, and urine calcium and phosphorus were lowered during the period between four and six months post-injury. Fat-free dry

weight and percent ash of injured and noninjured metatarsal bones demonstrated a true osteoporosis in the injured bone. Increased osteoclastic resorption, especially on the periosteal surface, and enhanced remodeling of trabecular bone were evident histologically. Microangiography showed increased vascularity in the affected areas at two weeks post-injury, while ^{99m}Tc scintiscan demonstrated hyperemia at two days post-injury.

The results of this study suggest cold-induced inflammatory hypervascularity with secondary osteoclastic resorption. A resorptive stimulus related to increased vascularity is postulated: a local factor that acts directly on the bone and/or enhances the sensitivity of the bone to normal circulating levels of parathyroid hormone is proposed as the mediator of the unusual osteoporosis that involves primarily the periosteal envelope.

CONTENTS

Chapter 1

STUDIES ON THE RESPONSE OF BONE AND SKIN BLOOD FLOW TO ACUTE COLD EXPOSURE: AN INTRODUCTION

People who inhabit areas that have cold climates learn to protect themselves against injury produced by low temperatures. In these areas, acute damage due to cold normally is limited to accidents involving skiers, mountain climbers, winter travelers, or the inebriate, unconscious in the alleys of large cities. In war, however, people are placed in circumstances where even the experienced are unable to take proper precautions. Armies have suffered as much from the cold as they have from the human enemy in time of war. Examples include Xenophon's Greeks in Armenia (400 B.C.), the Swedish troops of Charles XII in the Ukraine (1708), the army of Washington at Valley Forge (1777-78), and the forces of Napoleon fleeing Russia (1812-13). More than one million cases of frostbite were recorded in the two World Wars and the Korean War (1). The Israeli Defense forces suffered frostbite on the Golan Heights during the recent Yom Kippur War (2).

The pathophysiological responses of the body to low temperature and to the freezing of tissue are poorly understood, and the treatment of cold injury remains symptomatic. Most frostbite research has focused

on the response of soft tissue of the extremities to acute cold exposure. The studies described here were begun with the objective of looking at two areas of cold-induced pathophysiology that remain enigmatic.

The purpose of the first phase of the studies was to produce bone lesions in the hind limb of the domestic cat by subjecting it to mild frostbite injury and to evaluate the relationship between such lesions and the compensatory vascular alterations that follow. In the second phase of the study, the objective was to record cold-induced vasodilatation in the footpad of the domestic cat and to evaluate the hypothesis that arachidonic acid metabolites are a part of the mechanism of that phenomenon.

COLD-INDUCED BONE LESIONS IN THE DOMESTIC CAT

A small number of references are made in the literature to skeletal changes following acute or chronic exposure of the extremities to cold (3-8). The pathology generally is classified into several groups by time of occurrence following injury and apparent radiographic bony change. Vinson et al. (7) followed 100 frostbite victims of the Korean War, radiographically, for a maximum of 15 months post-injury. A classification of bone change produced by frostbite was divided as follows: (1) osteoporosis (the most common abnormality reported), (2) acromutilation (destruction of tips of digits following exposure of bone to air), (3) juxta-articular areas of decreased density (seen in individ-

uals who sustained 1st and 2nd degree injury, and first noted eight months post-injury), and (4) other bone changes (periostitis, new bone formation, and osteomylitis). The most common radiographic abnormality, osteoporosis, was often seen proximal, but not distal, to the area of injury, serving as an indicator of intact and altered circulation and metabolism. This radiographic lesion closely resembles those seen in disuse atrophy or microcirculatory disturbance in bone that cause negative calcium balance (9). The juxta-articular changes (7) resemble the bone erosions and subchrondral cysts seen in the later states of rheumatoid arthritis (8, 10). Similar changes have been reported, as "neurovascular" in origin, after chronic exposure to moisture and cold temperatures above freezing (6). These also included pathology of articular cartilage. Digital epiphyseal necrosis and clubbing after frostbite also have been reported (3, 4, 5, 8, 11, 12).

Although some investigators believe direct cold-injury to chondrocytes is important in bony changes following frostbite (3), most agree that primary circulatory changes are the key to such pathology in bone (5, 13, 14). Ervasti (13) has described acute circulatory changes in bone following cold injury in man as resembling those in adjacent soft tissue and has identified microscopic necrotic areas without nuclei in most of the bone samples studied. Specific laboratory findings in digital epiphyseal necrosis have been absent; cold agglutinins, cryoglobins, etc. have not been demonstrated. Sedimentation rates have been normal, and rheumatoid factors have been absent (3).

Attempts to reproduce the above-described lesions in animal models have met with little or no success (5, 14, 15, 16). Schatzki produced severe frostbite in one hind foot of 73 rabbits and studied them radiographically for up to 12 months post-injury (15). All animals lost soft tissue and bone (4th degree injury). The most common radiographic change was periosteal new bone formation seen at 1-2 months post injury in 82% of the rabbits. Seventy-five percent showed diffuse radiolucency (apparently osteoporosis) with the greatest incidence between 6 and 12 months post-injury. The lesions produced in this study varied significantly in type and distribution from those reported in man.

In 1957, Goldhaber showed that, in tissue culture, O_2 at 1 atmosphere promotes removal of bone, while reduced O_2 pressure promotes formation of bone (17). Formation of bone in tissue culture also was prevented when the pH was maintained at 8.0 rather than 7.0 (18). Currently, to assure a successful osteogenic preparation, 5% CO_2 in the gas phase of tissue culture generally is recommended (19). Chick embryos incubated for 10 days at 38° C in 3% CO_2 have 85% more calcium than eggs incubated in 100% O_2 (20). Richards and Brookes (21) showed elevated local CO_2 at the height of bone regeneration following experimental osteotomy in rabbits; further, venous congestion produced by femoral vein ligation has been shown to cause calcification of rat knee joint cartilage (20). The above data show clearly that increased PCO_2 may both stimulate osteoblastic differentiation and promote calcification.

The rat knee joint study (20) illustrated not only a rise in P_{CO_2}, but also a lowering of pH. Low pH and high levels of CO_2 also have been recorded in clinical osteoarthritis of the hip (22), osteoarthritic femoral heads (23), and impacted fracture of the femoral neck. All of these lesions exhibit sclerosis, positive calcium balance, and increased radiodensity.

The opposite phenomenon, i.e., alkaline pH and low P_{CO_2}, occurs in areas of increased blood flow and is compatible with decreased calcification and softening of bone. Examples of this occurrence include the spongy character of the normal metaphysis, which is a relatively alkaline area of the bone, and the lack of bone in the normal marrow, which is the area of highest pH in bone (20). The blood supply and pH increased during trabecular bone formation in experimental fractures; then flow fell below normal as calcification occurred. In another experiment, following ligation of the nutrient artery, cortical vascularization by periosteal vessels increased, leading to decalcification of the cortex (20).

Paget's disease results in a marked decrease of bone density and a characteristic decrease in production of spongy bone. Twenty-fold increases in blood flow (24) and pH readings of 7.31 have been recorded in such bone (20).

The data cited above support the hypothesis that sclerosis occurs and bone removal is decreased in an environment of low pH or low blood flow, and that bone removal is accelerated by high pH or increased

blood flow.

The purpose of this portion of the study was to induce cold injury in one hind foot of a group of cats, and then to evaluate the effect of that injury on bone. The following questions were to be considered during the course of the study:

(1) Can cold-induced bone lesions of the type described in man be produced consistently in laboratory cats?

(2) Is there evidence of local and whole body thermoregulation in the anesthetized cats during exposure to low temperatures?

(3) Can the juxta-articular "rheumatoid-like" lesions be produced in the cat?

(4) Do radiographic appearances correlate with lesions seen microscopically?

(5) Do microscopic lesions suggest death or altered activity of osteocytes, or altered calcium balance consistent with the blood supply to the area?

(6) Can ongoing bone pathology be detected via chemical determinations of serum and/or urine?

(7) Do angiographic visualizations of relative vascularity and/or bone scan data correlate with radiographic and biochemical indicators of ongoing pathology, eventual microscopic appearance, and microincineration findings?

(8) Is autoimmune activity present, supporting a possible relationship between cold-induced injury and rheumatoid bone disease?

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Chapter 2

THE DOMESTIC CAT AS A MODEL FOR THE STUDY OF COLD-INJURY

Classical frostbite typically occurs after rather brief exposure to severe cold and may result in loss of digits or entire extremities (1). Frostbite is a significant medical problem in subarctic areas of the world, including the northern tier states of this country. The mountain climber, careless skier, injured snowmobiler, or hunter are but a few of the potential frostbite casualties. The most susceptible population remains the military ground troops in time of war.

The exact pathophysiology of frostbite is unknown. Most investigators believe direct damage to tissues and secondary injury following vascular stasis are involved. The degree to which each of these factors contribute and the actual mechanism by which they operate remains unknown. Treatment of frostbite, though advanced during recent years, remains symptomatic (1, 2). Control of tissue loss and salvage of function constitute success. Human experimentation is prohibited by the devastating nature of the injury, and the study of clinical cases is limited by their relative infrequence in peace time. Furthermore, most frozen extremities are rewarmed by the victim or lay personnel long

before reaching definitive care facilities and the observation of health professionals.

BACKGROUND

Investigators have used animal models in attempts to understand the mechanisms of injury or to evaluate therapeutic regimens. Fuhrman and Crismen (3) summarized the methods of injury production in their classic studies of gangrene following cold injury. The methods include immersion in liquid, application of sprays, exposure to cold air, and direct contact with cold solids. They chose to produce an experimental injury by immersing the rabbit ear or foot in a mixture of ethylene glycol, ethyl alcohol, and dry ice for a fixed period of time at a specific temperature. Their goal was a reproducible injury for use in the study of the treatment of gangrene.

Sjostrom, Weatherley-White, and Paton (4) succeeded in producing a predictable lesion in the rabbit ear by using uniform time/tissue temperature curves rather than a uniform environmental temperature. This method was an improvement over earlier techniques.

Freezing in circulating liquid facilitates control of the tissue cooling rate because of the excellent conducting qualities of the medium; however, this characteristic may lead to too rapid freezing unless fluid temperatures can be raised during the exposure period. The use of dry ice or cold sprays such as ethylchloride or ether as contact coolants has been limited (5). Both of these methods make difficult

the control of the extent and the uniformity of injury.

Exposure of an extremity to moving cold air most nearly duplicates conditions under which frostbite usually occurs in man. Animal models have been developed utilizing the mouse tail (6, 7) and the monkey or rabbit foot (5, 8) suspended in a chamber through which cold air is pumped. Such a system allows one to control both air temperature and air movement; furthermore, temperature control is accomplished more easily than in a liquid cooling system.

The purpose of this work was to develop an animal model suitable for use in the study of the long term effects of cold injury on bone. Several criteria for the model were defined. (1) The animal must have physical and physiological properties similar to man, be readily available, and be economical. (2) Pharmacological restraint during production of the injury should not impinge seriously on normal vascular reactivity or thermoregulation. (3) The method of cooling must be realistic, and the degree of injury must be predictable and reproducible. (4) Rewarming and nursing care must be compatible with current procedures for human patient care.

The domestic cat was chosen as the animal to be used in these studies. The cat is the largest readily available and economical mammal whose anatomy and vascular physiology are similar to that of man. The small rodents are unsuitable because of their small tissue mass and the difficulty of obtaining adequate samples of their body fluids for biochemical evaluation. The cortex of the rabbit bone is less dense, which

makes radiographic examination more difficult. Furthermore, cold-induced bone lesions have been difficult to produce in that species (8). Subhuman primates require special housing and handling facilities and are prohibitively expensive.

Pilot work showed that bone lesions could be produced in the cat or the dog. The cat was selected because its peripheral vascular response to cold (i.e., peripheral control of cold-induced vasodilatation) is more like that of man than is that of the dog (9). The cat also is more easily housed and cared for than the dog. The one disadvantage of the chronic cat model is its susceptability to respiratory viruses and other contagious diseases. This problem can be minimized by a stringent program of preventive medicine.

Frostbite in man is painful just prior to freezing of tissues and during rewarming. Pain is most severe during rewarming and often requires analgesia. Humane practice and restraint required for instrumentation of animal models demand general anesthesia during the production of the injury and rewarming. Pentobarbital Na and Ketamine HCl were tried. Pentobarbital Na was selected as the agent because of ease of administration, adequate duration of action, and minimal effect on the thermoregulatory system of the animal. Pilot studies demonstrated a catecholamine-like effect of Ketamine HCl, which totally blocked cold-induced vasodilatation.

MATERIALS AND METHODS

Adult domestic cats were acclimatized for 7 to 10 days at 21 to 24° C. The cats were anesthetized with pentobarbital Na (30 mg/kg, IV). Their hind feet were shaved to the level of the hock, and their metatarsal footpads were cleaned with isopropyl alcohol. Needle thermocouples (23 g x 3/4 inch) were positioned within the foot so that the recording tips lay between the metatarsal footpad and the distal epicondyle of 3rd and 4th metatarsal bone. Copper-constantan bare-wire-loop thermocouples were held in place on both metatarsal pads and insulated slightly by two layers of plastic adhesive. A coated thermocouple was placed in contact with the colonic mucosa by insertion through the anus. Footpad surface, deep foot tissue, and rectal temperatures were recorded on a strip-chart recorder. Footpad temperatures were recorded to monitor cold-induced vasodilatation of surface vessels, deep foot temperatures to gauge injury production, and rectal temperatures to assess changes in core heat balance. Both hind legs were suspended through holes in a cotton hammock, supporting the animal without inhibiting normal blood flow in the extremities (Figure 1). The interior of a styrofoam chamber (13 x 14 x 24 cm) was precooled to -50° C and one foot was suspended through an opening in the top of the chamber to the level of the stifle joint. Compressed air was dried in silica gel, then cooled by passage through a copper coil (4 m x 0.4 cm ID) suspended in a second styrofoam chamber filled with liquid nitrogen. The temperature of the freezing chamber was maintained at 50° C ± 5° C

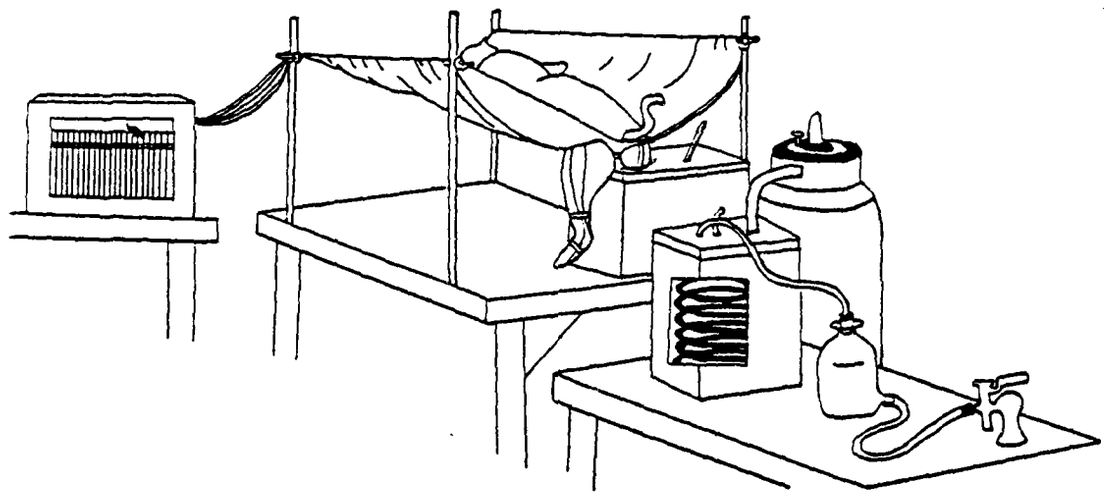

Figure 1. Apparatus for production of cold injury; from right to left, compressed air source, vacuum bottle containing desicant, copper coil in chamber of liquid nitrogen, freezing chamber with cat suspended from above, and temperature recorder. Liquid nitrogen source stands behind the table in the foreground.

by altering the rate of air flow through the copper coil. Typical air flow was approximately 70 $cm^3 sec^{-1}$.

The duration of exposure was variable, as the criterion for exposure was deep foot temperature reached, not time of exposure. The foot was rewarmed in a 41-42° C water bath immediately after removal from the cold chamber. Thermal layering in the water bath was avoided by moving the foot slowly from side to side during rewarming. This technique has been shown to be as effective as whirlpool rewarming (10) and is the method likely to be used in the field. Following rewarming, the feet were dried and the cat was placed between insulating pads to recover from anesthesia. A warming lamp was suspended above the recumbent animal to facilitate the return of depressed core temperatures. A protective Robert-Jones bandage was applied to the injured foot before returning the animal to its cage. Injured feet were suspended in a 38-40° C "whirlpool" bath of povodine iodine solution for 10 minutes daily. Protective bandaging was replaced daily until edema subsided and vesicles dried, in the case of superficial injury, or until demarcation, loss of necrotic tissue and adequate granulation occurred in deep injury. Debridement of portions of toes, when necessary, was accomplished under general anesthesia.

RESULTS

Metatarsal pad temperatures dropped to zero or slightly below within four to seven minutes after exposure to cold air. The initial

drop in surface temperature was followed by cyclic or prolonged warming of the footpad following the patterns described by Schwinghamer and Adams in their study of cold-induced vasodilatation in the cat (11). When the initial footpad temperature dropped only slightly (i.e., ≈ 10 to 15° C) before cold-induced vasodilatation occurred, there followed a modest rise of 5-7° C and then a sudden plunge toward the temperature of the freezing chamber. When pad surfaces blanched and froze quickly after exposure, their temperatures dropped to -10° to -20°, then usually rebounded vigorously and remained near to or above zero for 25 to 180 minutes (Figures 2 and 3). Cold-induced vasodilatation eventually failed in all animals and metatarsal pad temperatures dropped toward the ambient temperature. When this occurred, pad surfaces blanched and then became frosty white and firm to the touch. Transient blanching and frosty appearance also occurred between cycles of cold-induced vasodilatation.

Deep foot tissue temperature dropped from 37-38° C to approximately 30° C during the first five minutes of exposure to cold and fluctuated with cycles of cold-induced vasodilatation. Individual deep foot temperature cycles were not always superimposable over the footpad cold-induced vasodilatation, but general similarities in the patterns were seen. Following the halt of cold-induced vasodilatation, deep foot temperatures plummeted within 3-5 minutes. Deep foot temperatures often dropped so rapidly at this point (30-50° C min^{-1}) that it was difficult to transfer the foot to the warm water bath before the temperatures had

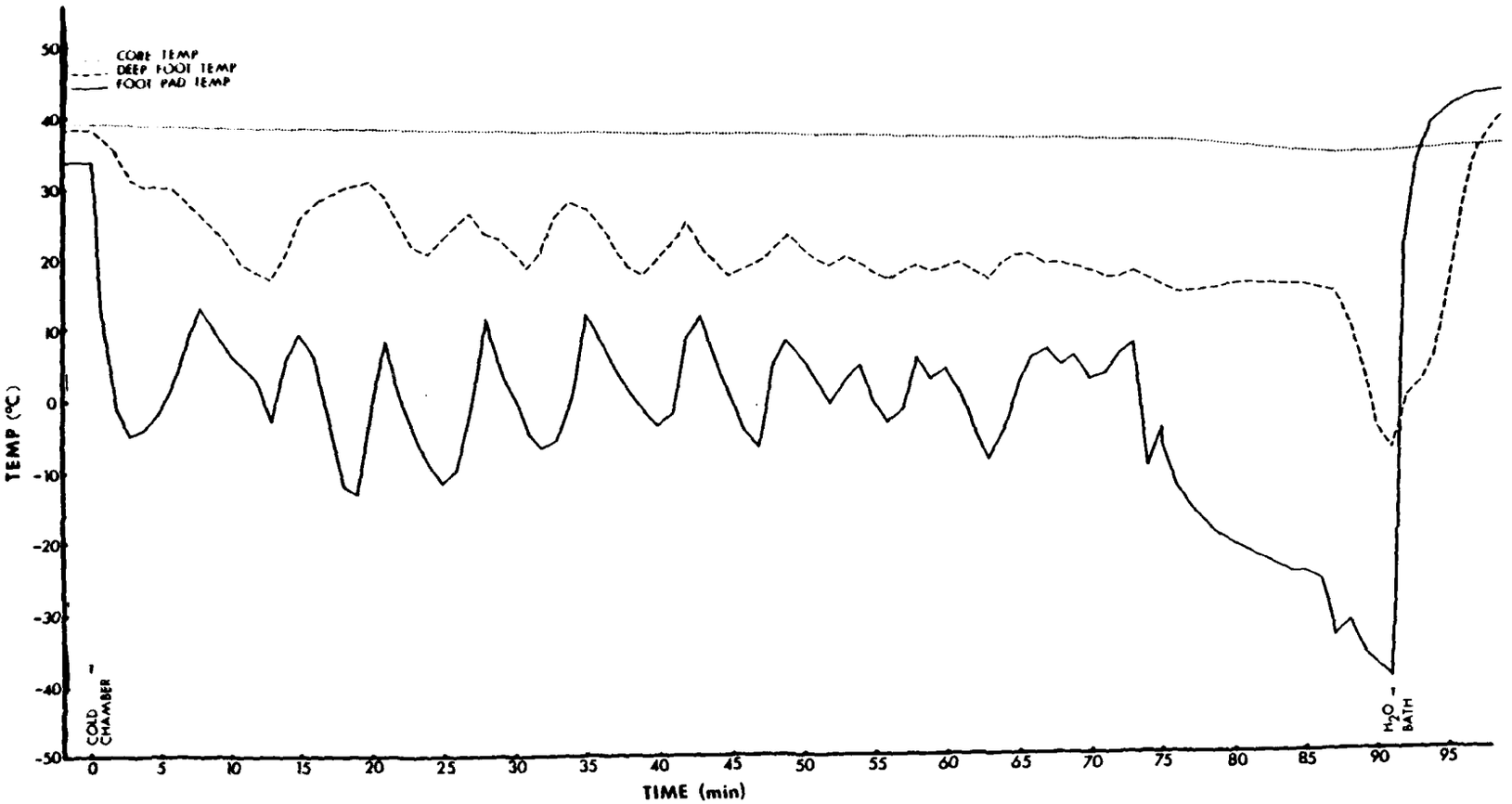

Figure 2. Time-temperature curves of rectal (dotted line), deep foot (dashed line), and footpad surface (solid line) temperatures during production of cold injury in the foot of a cat. The time of cold exposure was approximately 91 minutes. (Cat #26.)

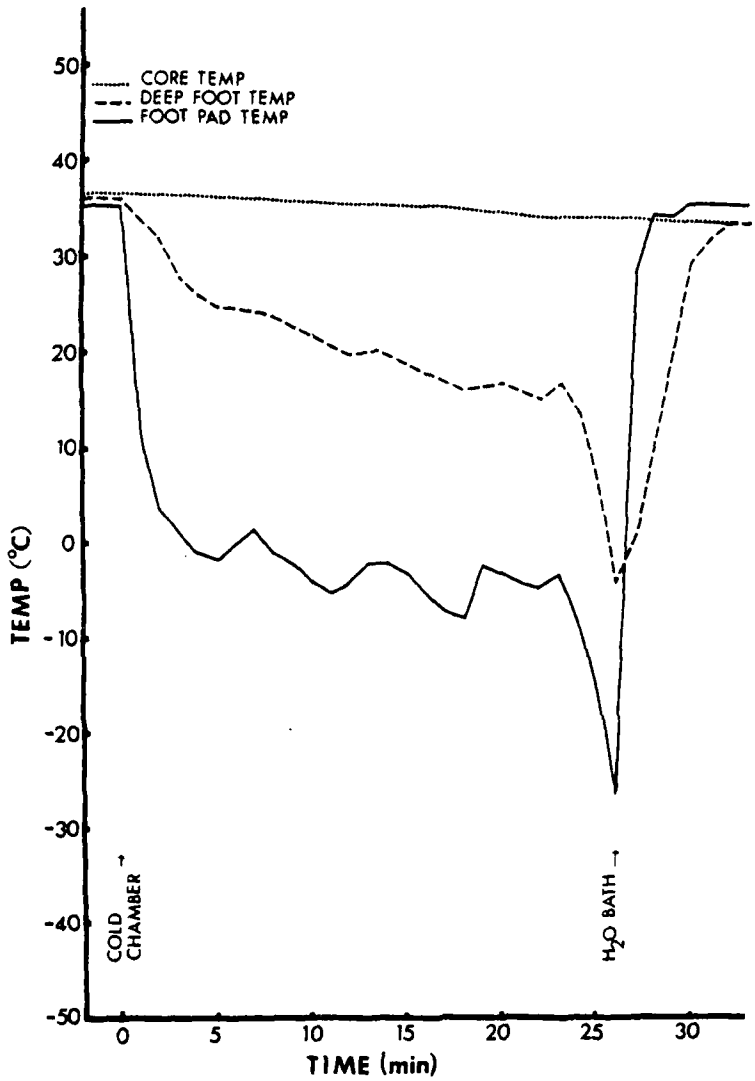

Figure 3. Time-temperature curves of rectal (dotted line), deep foot (dashed line), and footpad surface (solid line) temperatures during production of cold injury in the foot of a cat. The time of cold exposure was approximately 25 minutes. (Cat #9.)

reached -30 to -40° C.

Rectal temperatures dropped 1-2° C within the first 10 minutes of exposure, then stabilized and decreased more slowly throughout the remainder of the cooling period. Reduction of the core temperature during cold exposure increased with time of exposure. The time of exposure increased with the magnitude and duration of the cold-induced vasodilatation response, as the latter delayed onset of deep tissue freezing, the criterion for removal of the foot from the cooling chamber. Total time in the cooling chamber varied from 3 to 218 minutes. Time of exposure did not correlate with degree of injury.

After removal from the cooling chamber, the frozen toes were hard to the touch and frosty gray in appearance. In all cases, digits 3 and 4, the metatarsal pad, and the dorsal skin over the distal metatarsals 3 and 4 were frozen. In several animals, digits 2 and 5 remained pliable and their pads were pink at the time of removal from the cold chamber. Rewarming to preinjury temperature required from 3 to 7 minutes in the 41-42° waterbath.

A bright pink color replaced the pallor of the footpads as the foot became pliable in the rewarming bath. Footpad temperatures continued to rise 2-4° above preinjury temperatures after active warming ceased. Even in footpads that would become necrotic later, the hyperemia persisted during a post-thaw period of approximately 45-60 minutes; in most animals, the hyperemia persisted until bandages were applied at 2 hours post-thaw. Local edema began immediately post-thaw and was

marked by two hours post-thaw. Edema extending proximally to a definite line between the base of the digits and midshaft of the metatarsal bones caused extension and splaying of digits. Edema of the area that actually was frozen reached maximum within the first several hours post-injury. More proximal edema, often extending to the stifle joint or above, was first noted 12 to 24 hours post injury and remained for 2-5 days. Injuries almost severe enough to produce tissue loss caused the greatest amount of edema.

Skin vesicles like those seen in man were first noted at 1-2 hours post-thaw; in a few animals, vesicles were not seen until the bandage was removed for the 24 hour post-injury treatment. The size of the vesicles varied from 4 to 10 mm in diameter. Vesicles were seen only in interdigital spaces, more commonly on the plantar surface than the dorsal surface. Vesicles contained either a clear or a serosangenous fluid; in retrospect, serous vesicles were a good prognostic sign, while bloody vesicles reliably indicated a more severe injury.

A typical mild (1-2 degree) injured limb was edematous to the level of the hock, warmer to touch than the contralateral foot, and had bright pink pads and large clear interdigital vesicles one day post-injury. The edema and hyperemia subsided during the next 3-7 days and the vesicles ruptured on about day 4. The protective bandage was unnecessary at 5-6 days post-injury. An animal that would later lose soft tissue or bone (3-4 degree injury) might show slightly less edema than the superficially injured animal. As with mild injuries, the damaged

foot was warmer than the contralateral one. Still hyperemic but doomed, toes or pads became cyanotic and cooler to touch than the other digits on day 1 or 2 post-injury. Vesicles, when present, were slightly smaller and contained serosangenous fluid. Clear demarcation by skin color was not evident until day 4 to 5. The skin separated at the demarcation line about 7-10 days post-injury. Edema subsided more rapidly in severely injured portions of the foot, and skin often took on a white or "fishflesh" appearance just before separation. When soft tissue sloughed, amputation of phalanges become necessary; this was done at approximately 12 days post-injury. Following amputation or loss of skin, rapidly advancing granulation tissue covered the wound within a few days. The animals ignored the frostbite lesions to the point that the moist lesion collected hair, litter, and debris from the cage floor, necessitating application of a protective bandage until healing was complete. Misshapen toe nails and loss of pigment in previously colored hair were seen at 2-3 months post-injury in feet that sustained 3rd and 4th degree injuries.

DISCUSSION

The cold injury dose/response ratio for frostbite in man is extremely variable. Only 10 soldiers in 100 may suffer injury, though all are exposed to the same environment. Even among the injured ten, several may suffer only passing edema of digits or lose epithelium from the pinnae, while one or two may lose entire extremities. Variation is attributed to the utilization of protective clothing, degree of activity,

state of nutrition and hydration, a thermovascular factor, degree of fatigue, mental attitude, and most important — knowledge and "cold discipline." With the exception of the thermovascular factor, all of the variables mentioned can be controlled through training, discipline, and esprit de corps. The vascular-thermoregulatory factor is sometimes negatively controlled in man by tight boots or clothing. The cat model described here permits expression of individual thermoregulation by eliminating tourniquet or vasoconstrictor drugs.

One group of 9 animals that sustained nearly identical 1st degree injuries experienced minimum deep foot temperatures between +6 and -1° C, yet total exposure time varied between 21 and 218 minutes. This wide variation of exposure time necessary to produce a given injury apparently is a direct result of variation in ability to thermoregulate through local vascular and whole body mechanisms. Cold-induced vasodilatation was seen in all animals studied, and shivering thermogenesis was a visible part of thermoregulation in many of those that required a long exposure to the cold. Cold-induced vasodilatation of the surface vasculature, which is rich in A-V shunts, appears to provide a "wall of fire" protecting the tissue beneath. As long as cyclic pulses of warm blood circulate through the superficial tissues, a "physiological glove" is provided, which prevents excessive loss of heat from the deep tissues. Vasoconstriction "removes the glove" which increases loss of heat from the deep tissues. This hypothesis is consistent with the delayed manner in which deep temperatures follow sur-

face temperature.* Core heat appears to be the currency that buys the gloves, while individual differences in manufacturing, regulating, or distributing core heat may explain major variations in the duration of exposure necessary to produce a given degree of injury. A detailed discussion of and hypothesis for the mechanism of cold-induced vasodilatation in the domestic feline has been described (12).

The peripheral thermoregulatory capability of the individual subject is paramount in the resistance or susceptability to frostbite in man. The ability of the pentobarbital anesthetized cat to demonstrate what appears to be intact peripheral thermoregulation makes this model extremely suitable for the study of the mammalian response to a cold environment.

The fact that digits 3 and 4 froze before digits 2 and 5 can be explained only by variation in vasculature. Digits 2 and 5, the ones most likely to be exposed to trauma, appear to possess a better surface blood supply than do the two middle digits. Similar patterns for tissue loss were seen in the tourniqueted rear limb of the dog cooled in liquid, though the entire foot was frozen solid in that model (10). A superficially injured dog foot typically lost tissue only on the dorsal surface of digits 3 and 4. Physical principles of mass, exposure, and convective heat loss would lead one to expect the central digits to be

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* In 5 animals evaluated, a positive correlation was seen between footpad temperatures and the deep foot temperatures one minute later ($r = 0.73$).

more resistant; microangiography supports the vascular hypothesis.

The dog can maintain foot temperatures above freezing at the expense of core heat, for extended periods in a liquid environment of at least -30° C. The cat maintains foot temperature for only 30-45 minutes in an ice water bath before peripheral vasoconstriction occurs and foot temperature drops to that of the bath. Dogs, whose feet froze only when the blood supply was halted by a tourniquet, sustained deep foot temperatures of -30° C for 15 to 30 minutes without subsequent tissue loss (1st degree injury). In the cat, a similar injury resulted when the foot was rewarmed immediately on reaching 0° C. Nine cats that sustained tissue injury severe enough to cause the loss of at least 2 phalanges on one or two digits (digits 3 and/or 4 in each case) were cooled to sub-zero temperatures for 2.5 to 6 minutes. Among this group, neither time below zero nor temperature reached correlated with degree of injury, though each reached at least -8° C. The species variation in resistance to cold-induced tissue damage has not been explained.

As in the dog, the time frame of post-injury pathogenesis in the cat is compressed when compared to man. This is an advantage of the animal model, which allows a larger number of injuries to be examined during a given period of time. The following times may be considered comparable: maximum edema 1-2 hours (cat), 36-48 hours (man); vesicle rupture 4-5 days (cat), 4-10 days (man); eschar separation 7-10 days (cat), 20-30 days (man). Vesicle formation in the cat is more like that of the dog than that of man. The animals demonstrate vesicles in

the interdigital spaces, while in man, vesicles are seen over the dorsal surface of the fingers, beginning just proximal to the nails and extending over the dorsum of the hand. This species variation may be due to histological differences in the skin.

Using the above described methods, one may produce consistent superficial or deep frostbite injuries in the cat. Superficial (1st to 2nd degree) injuries will be produced when feet are exposed to the -50° C air environment until deep tissue temperatures reach 0° C. Allowing the foot to remain in the cooling chamber for 5-7 minutes after deep temperatures reach zero will result in a deep (3rd to 4th degree) injury. During the 5-7 minute freeze period, tissue temperature reached and rate of temperature fall will vary, but in the cat these factors appear to be less important than the period of time spent below zero. Using the above described methods, one might expect 75-80% success in predicting the degree of injury. The 20-25% failure rate might be reduced further by careful placement of the deep thermocouple, including radiographic verification. Placement of the thermocouple tip more proximal or near a major vessel will cause more severe injury than expected, much as installing a thermostat near a heat source will cause the temperature to fall in a far corner of the room.

The literature on frostbite is replete with painstaking modeling of injury production, yet without any thought for post injury nursing care. Nursing care is a major factor in the successful modeling of frostbite injury (10). The Robert-Jones protective bandage is probably

less important in the cat than the dog. Though neither species appears to suffer discomfort following injury, the dog begins chewing on the injured extremity 3-5 days post injury. The dog also is less careful than the cat to avoid his own excrement, resulting in exposure of the limb to cage filth. However, the bandage also is useful in the cat. Because of apparent loss of sensory nerve supply after injury, the cat ignores the injured limb, failing to groom it as expected. Until the wound is healed and dry, cat box litter and hair from the cage floor adhere to the lesion, retarding healing. The Robert-Jones bandage precludes this problem. Whirlpool therapy serves several purposes in the treatment of the frostbitten cat foot. Skin sustained by a compromised vasculature readily supports bacterial invaders such as Pseudomonas sp. The daily soak in warm water that contains povodone iodine facilitates control of such organisms. Secondly, the gentle massaging action of warm water cleans and debrides sloughing soft tissue effectively without trauma. Finally, the pulsating warmth may stimulate vasodilatation or even enhance vascular return to the compromised area. It is important to avoid overheating of tissues; while slight increases in temperature may be therapeutic, great increases may influence the local metabolic rate and place increased demand on an already overworked blood supply.

SUMMARY

The domestic feline, anesthetized with pentobarbital Na, is a

suitable model for the study of frostbite or its treatment and for the study of mammalian physiological responses to cold. The cat, injured in a cold air environment, shows both peripheral and core temperature regulation. Methods of consistent injury production and proper nursing care are described. Lesions that result are described and similarities to frostbite in man are explained.

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Chapter 3

A RADIOGRAPHIC EVALUATION OF COLD-INDUCED BONE LESIONS IN THE CAT

Changes in calcified tissue following acute cold injury have been detected radiographically in man (1-8). The pathophysiology of these lesions has not been described, nor have the lesions been reproduced successfully in animal models (9-11). Schatzki reported periosteal new bone growth and diffuse loss of density in the feet of nearly all of 73 rabbits after severe experimental frostbite (10). The periosteal proliferation and new bone growth seen in that study appear excessive when compared to radiographic studies and reports of cold-induced bone pathology in man.

The purpose of this study was to induce skeletal pathology in the hind feet of cats by exposure to low temperature. Pilot work showed that the lesions produced, unlike those seen in previous animal studies, would typify the changes seen in man after cold injury. Serial radiographic studies might uncover the chronology of the lesions while bone scans and terminal microangiography should help elucidate vascular changes that occur in conjunction with the pathology of bone.

MATERIALS AND METHODS

Thirty conditioned cats 8-14 months of age were acclimated for 7-10 days at 22° C. Pilot radiographs were made of both hind feet. Ten of the 30 animals were noted to have open metatarsal epiphyseal lines; all of the other animals were mature. The right hind foot of all animals was frozen in cold moving air by the method described previously (Chapter 2). Cold injuries were classified in retrospect, using the traditional system; six animals sustained 1st degree, 2 sustained 2nd degree, 8 sustained 3rd degree, and 14 sustained 4th degree injury. Following exposure to cold, the animals were maintained for up to 6 months and studied by radiography at monthly intervals. Radiographs were made with standard clinical equipment and high contrast industrial non-screen film. All processing was done by hand.

Thirteen animals were studied by microangiography at selected intervals following injury, using the technique of Erol et al. (12) slightly modified (Figure 1). Under pentobarbital anesthesia, the abdominal aorta was cannulated at the level of the renal arteries. Animals were perfused at 130 mmHg pressure first with Micropaque^{R} in heparinized saline, then Micropaque^{R} in 10% formalin (300 mg/l). The formalin infusion was started when the barium in saline effluent from the jugular vein became white; all perfusion was stopped when muscular twitching induced by the formalin ceased. Rubber band tourniquets were placed on the hind feet above the hock, and the feet were amputated and stored in 10% formalin under refrigeration for 48-

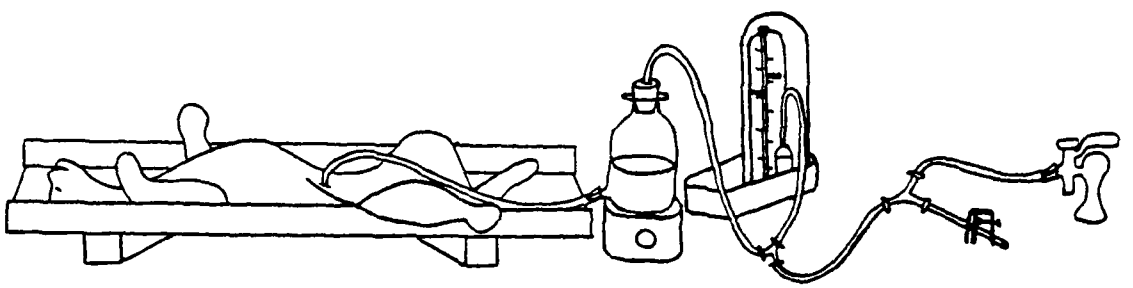


Figure 1. Drawing of microangiography perfusion apparatus. From right to left, compressed air source, "Y" with hose clamp for pressure bleed-off adjustment, manometer, vacuum bottle on magnetic stirrer, and animal being perfused.

72 hours. The bones were cleaned of soft tissue and periosteum and decalcified in formic acid (13). The decalcified feet were radiographed using a Hewlett-Packard Faxatron^{R} soft x-ray unit and non-screen film.

Contralateral feet were used as controls.

Several animals were studied at selected pre- and post-injury periods by bone scan, utilizing ^{99m}Tc as a marker (14). Six mCi of ^{99m}Tc tagged 1-hydroxyethyldene diphosphonate

was injected into the cephalic vein 3 hours before scanning. Animals then were anesthetized with Ketamine HCl. Counts were performed on a Nuclear-Chicago Pho/gamma HP unit.

RESULTS

a. Radiographic and microangiographic findings

Except in the instance of 4th degree injury, in which entire phalanges sometimes were lost, initial degree of tissue injury was unrelated to later radiographic changes. Radiographic observations were classified into the following categories. (1) No lesion — six of the 30 animals demonstrated no discernable changes in bone. (2) Apparent demineralization — nine feet showed a "lace like" loss of cortical density, six showed permiative or "moth-eaten" demineralization, one showed marked cortical thinning, and five had generalized areas of bone demineralization with accentuation and alteration of the trabecular pattern. Seven animals had diffuse loss of density, and two of those showed decreased cortical definition. (3) Other lesions — three young animals showed

early epiphyseal closure in injured metatarsal bones. Four animals had loss of density or resorption of articular surfaces, and two showed periosteal proliferation or new bone growth. Two cats showed localized juxta-articular loss of density. Finally, five animals showed isolated sclerotic changes, and four had lytic areas of bone, probably as a result of secondary osteomylitis.

The feet of thirteen injured animals were examined by microangiography. One showed no change, one showed minute areas of extravasation of contrast media, and eleven showed increased vascularity; five of the latter exhibited what appeared to be pooling of contrast media in the medullary space.

The most common radiographic lesion was a mottled, lacy, or diffuse loss of cortical density, often seen in the first phalanx of the digits and usually detected first at 2 to 3 months post-injury (see photos 1a-b and 2a-b). Lesions of this type were seen in all four degrees of frostbite injury. One animal that had sustained a 2nd degree injury showed generalized loss of radiodensity and an accentuation and disruption of the trabecular pattern beginning at 1-2 months post-injury (photos 3a-g). Medullary vessels were increased in number as early as one month post-injury in animals that suffered demineralization of metatarsal or phalangeal bones. Premature closure of the epiphyses was seen as early as 2 months post-injury (photos 4a-d, 5a-j). Note the apparent decrease in metaphyseal blood supply and pooling in epiphyseal areas in the metatarsals that show early closure (photo 4e). Articular

surfaces generally were spared, although several animals showed resorption or roughening of articular surfaces (photos 6a-c), and one animal showed an ankylosed metatarsal-phalangeal joint following a severe injury (photo 7c). Photos 5j and 7c show juxta-articular punched-out lesions, although they are not as distinct as the lesions described by Vinson (5), and do not appear to include joint cartilage. Periosteal proliferation is shown in photos 8b and 9b. One of these animals showed pooling of contrast media within the proliferative area (photo 8c), while a general increase in vascularity in the involved digit was seen in the other (photo 9c). Four animals showed severe lysis of bone (photos 10 a-h) beginning at 2 months post-injury. Two of these animals had draining tracts near the lytic lesion and apparently were suffering from a secondary osteomylitis following severe injury and resultant soft tissue necrosis. Sclerotic changes were rare and often indistinct but usually were seen as isolated lesions (photos 8b-c) with some evidence of altered blood supply surrounding the lesion.

Vascular changes were seen as early as two days post-injury (photos 11a-b), when minute extravasations appear to indicate increased fragility of metatarsal vessels. By one month post-injury, medullary vessels of metatarsal bones were increased in number. Vessels of the injured phalanges were tortuous and enlarged as well as increased in number (photos 6c, 8c, 9c). Skeletal changes seldom were seen by radiograph before 2-3 months post-injury.

b. Bone scan findings

One animal (photos 12a-i), which ultimately lost 3 toes and suffered general lacy demineralization of the remaining digits and metatarsals, showed a marked increase in ^{99m}Tc uptake on day two; the injured foot continued to show more uptake than did the control foot through 6 months post-injury. The toes that would be amputated later showed no uptake in bone at day two, yet all footpads were pink and appeared to be perfused adequately at that time. A general increase in intensity of ^{99m}Tc uptake was observed at approximately 2 months post-injury and persisted until 6 months post-injury. Photos 13a-d illustrate a series of scans of an animal that received a very mild 1st degree injury. The metatarsal region shows increased uptake at 2 days, and the entire foot shows an increase at 2 weeks and 1 month post-injury. Photo 14, a single scan taken at two months post-injury, shows a very slight decrease in uptake in the distal portion of the injured foot. Radiographs showed loss of cortical definition, general lacy demineralization, and pooling of contrast media in the digits at 3 months post-injury. Finally, photos 15a-b show a slightly decreased uptake in the toes at 2 months and a decrease in the metatarsal-phalangeal junction at 4 months post-injury. This animal had a general loss of density of metatarsal and phalangeal bones. Trabecular accentuation and reorganization was visible radiographically at 2 months post-injury.

DISCUSSION

The type and distribution of radiographic lesions seen in this study correlate well with reported cold-induced bone lesions in man. Like the study of 100 Korean War frostbite casualties (5), loss of radiodensity in various patterns was the most common lesion seen, while periosteal proliferation was relatively rare. These findings contrast with those of Schatzki, who reported periosteal new bone formation in more than 82% of the rabbits studied (10). The major variation in the type of lesion seen in the present study vs those seen in man is the nature of the juxta-articular lesions. In the cat, joint changes were less distinct and did not appear to invade the joint cartilage unless open lesions in soft tissue predisposed to secondary osteoarthritis. The juxta-articular lesions reported by Vinson were thought to be subchondral, with joint surfaces becoming involved only after 12-14 months, as a result of loss of structural integrity and secondary breakdown of joint cartilage (15). Radiographic loss of density seen in this study was similar to that reported in man. The time course of the radiographic lesions and the degree of injury required to initiate the lesion in cats were analogous to those in man. The osteoporotic lesions seen in man were no longer evident at 4 years post-injury. Table 1 compares the lesions noted in this study with those seen in man by Vinson and those seen in the rabbit by Schatzki (5, 10).

Contrast media pooled within the medulla in phalanges of digits that showed endosteal scalloping or major architectural changes. The

Table 1. Degree of injury and radiographic lesions noted in this study compared with those reported in man and the rabbit model study. Numbers represent percent of total individuals evaluated.

	Vinson (5) (100 men)	Present study (30 cats)	Schatzki (10) (73 rabbits)
degree of injury			
1st	3	20	0
2nd	10	7	0
3rd	70	27	0
4th	17	46	100
diffuse radiolucency	58	73	75
no lesion	36	20	0
lysis of bone	10	13	no data
juxta-articular lesions	7	7	1
periosteal proliferation	2	7	82
early epiphyseal closure	NA	30% of open	no data

pooling (photos 1c, 2c, 4c) showed "feathered" rather than distinct borders and appeared to result from extravasation rather than aneurysm-like increases in vascular lumen size. Brookes (16) was unable to explain similar findings in rat femurs after ligation of femoral veins and the venous congestion that followed. The findings resemble a post-traumatic aneurysmal bone cyst (17), in which an area within a metaphysis of a long bone behaves like a sponge, absorbing blood slowly from the bone capillaries and the periosteal vascular network. The cavities of the aneurysmal cysts lack distinct boundaries or endothelial lining. The pooling of contrast media accompanied endosteal scalloping in the cats studied here, and was not related to differences in perfusion pressure in injured and noninjured feet. In general, increased vascularity was seen in bone that showed loss of radiodensity.

Early epiphyseal closure has been reported in children after frostbite (1-8). Bigillow (8) states that injury in man must be of at least 3rd degree to cause premature fusion or destruction of epiphyses. Early closure of epiphyses was seen in 2nd (photos 3a-g), 3rd (photos 4a-e), and 4th degree injury in the present study. The only foot that showed early closure and was perfused (photo 4e) lacked the fine metaphyseal vascular network, normally visible well beyond the time of closure of the plate. Apparently, the cartilage growth plate became calcified after the rich vasculature in the area was disrupted.

Articular cartilage generally was spared (photos 10c-d), even when lysis occurred in large segments of bone. Joint cartilage differs

from bone primarily in that it is not mineralized and is apparently not susceptible to osteoclastic resorption; both differences might be explained by the presence of glycoproteins in cartilage. Articular cartilage receives its nutrition from sinovial fluid rather than directly from blood vessels as bone and epiphyseal cartilage do. If chondryocytes were injured directly by freezing, one would expect pathology of articular cartilage, which is one diffusion barrier further from its nutritive blood supply than bone or epiphyseal cartilage. If a primary vascular alteration is essential for tissue cell damage, it is not surprising to see sparing of articular cartilage after cold injury that affects both bone and epiphyseal cartilage.

Subchondral radiolucencies seen in this study were similar to early juxta-articular changes reported in man by Vinson. Lesions in the cat (photos 5j, 7b-c) showed increased radiolucency of subchondral bone at two months post-injury, but articular cartilage remained intact. The most notable changes in human patients occurred at 5-24 months post-injury, though articular cartilage was eroded as early as three months in some cases (3, 5). In a four year follow-up study of Vinson's 100 patients, Schatzki (15) noted that the number of individuals who had juxta-articular joint lesions increased to 19 from the original 7. The other major difference between these lesions in man and the cat is the radiographic appearance of the joint changes; the human lesions generally were small and had distinct borders, while the lesions in the cats were relatively larger and more diffuse. The etiology of these lesions has

not been explained.

Both instances of cats with periosteal change were unlike those seen by Vinson. The lesions in man simply showed a dense thickening of the outer surface of the cortex, while the lesions in cats appeared to be the result of vesicle-like elevations of the periosteum with calcification continuing beneath the raised membrane. The lesions reported by Vinson were no longer visible 4 years post-injury (15). The mechanism of formation of these periosteal alterations has not been explained, but microangiography of one cat (photo 8c) suggests a similarity to the post traumatic aneurysmal bone cysts discussed previously.

Sclerotic changes noted in this study were localized rather than involving whole bones. Whether osteopetrosis, whole bone sclerosis, was present at any time post-injury cannot be judged from the radiographs. Sclerotic changes were seen too seldom to support any hypothesis about their origin.

The exact interpretation to be put upon increased uptake of radionuclides in bone has not been established. Most investigators agree that increased blood flow and/or incorporation into immature bone enhance the uptake of ^{99m}Tc (18-21). The relative importance of blood supply vs osteogenesis in nucleotide uptake is still in question. Photos 12a-i show increased uptake immediately post-injury in all but the lateral distal portion of the injured foot. Although not clinically discernable at two days post-injury, the 3rd, 4th, and 5th digits would eventually become ischemic and gangrenous. This finding demonstrates

the potential of scintillation scanning in early evaluation of vascular injury. Uptake appears to be further enhanced at 2 months post-injury. The generally increased uptake throughout the testing period may be due to increased hyperemia in the foot, while the burst of activity at two months post-injury could indicate the start of enhanced osteoblastic activity. Photos 13a-d depict the scans of an animal that sustained a mild injury. All but the first 2 day post-injury scan show increased uptake in the injured foot. The decreased uptake at 2 days in the injured foot may be the result of edema and increased compartment pressures in that area, resulting in temporary ischemia. The animal shown in photos 15a-b appeared radiographically to be undergoing massive remodeling as early as 2 months post-injury, yet tracer uptake in the injured foot was slightly below that of the contralateral control. Scintillation scanning in these studies appears to serve as a more consistent indicator of vascularity than of osteoblastic activity. To distinguish between these two factors is difficult as they often go hand in hand during inflammatory, traumatic, or metabolic disturbance of bone metabolism (22).

Bone vascularity after frostbite in laboratory animals has not been studied previously. The data collected during the first month post-injury indicated that medullary vessels suffered abnormal loss of integrity as early as two days post-injury (photos 11a-b). New vessels were seen at one month post-injury in frostbitten feet. Increased ^{99m}Tc uptake seen in bone scans suggests general hyperemia of bones before there was radiographic evidence of decalcification. Clearly, hyperemia of soft

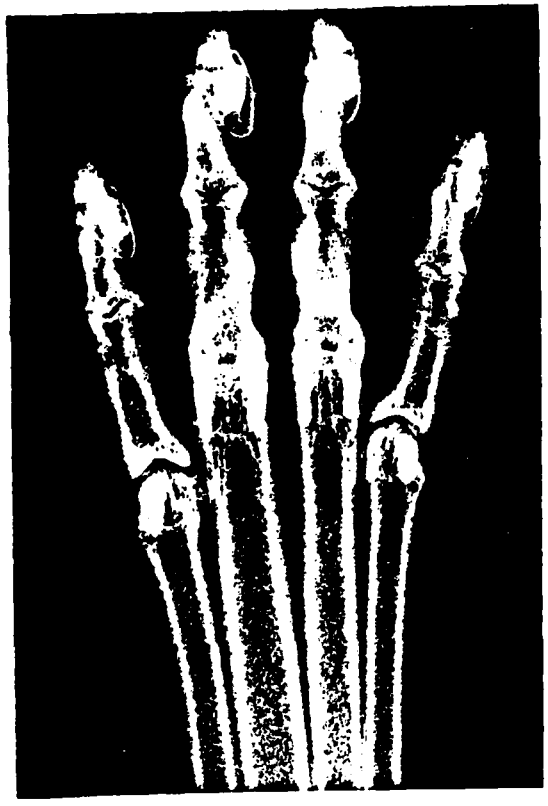
tissue occurred before radiographic changes in bones occurred. It appears that formation of new vessels occurred before calcified tissue changes were evident on radiograph. However, these data do not demonstrate unequivocally that vascular lesions preceded all skeletal change, as 30-50% of bone substarce must be removed before osteoporosis can be noted on clinical x-ray (14).

SUMMARY

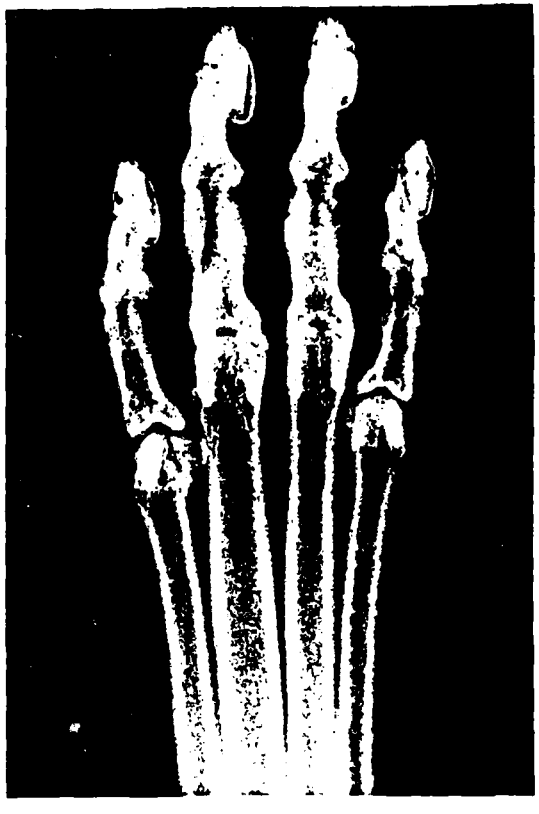
Experimental frostbite was produced in one hind foot of each of 30 domestic cats. The feet were evaluated by radiography, microangiography, and ^{99m}Tc bone scan for up to 6 months post-injury. Twenty-four animals showed skeletal lesions that included diffuse and localized loss of density, early epiphyseal closure, and periosteal proliferation. Bone scan and microangiography demonstrated hyperemia and increased vascularity of affected areas. Results support the hypothesis that cold-induced bone lesions occur as a result of primary vascular alteration.

Photo 1a. Radiograph, right foot, pre-injury. (Cat #32.)

Photo 1b. Radiograph, right foot, 2 months post-1st degree frostbite. Slight loss of cortical definition and diffuse loss of density of 1st phalanx of digits 2 and 5. Diffuse loss of density of distal metatarsals. (Cat #32.)

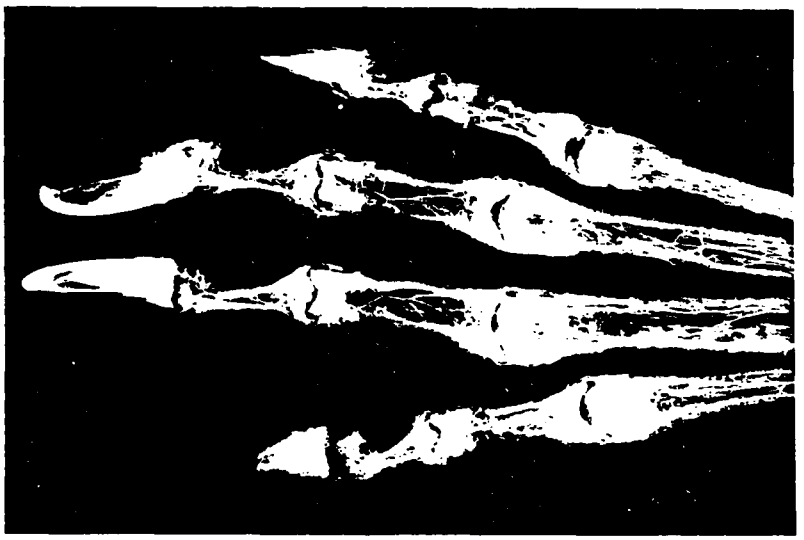


1a



1b

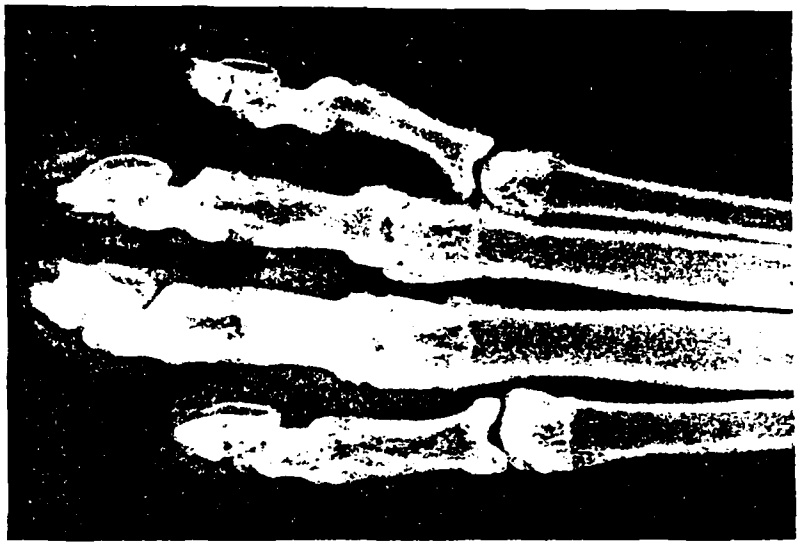
Photo 1c. Microangiograph, right foot, 2 months post-1st degree frostbite. Increased vascularity in metatarsals and 1st phalanx of digits 2 and 5. "Pooling" of contrast media in 1st phalanx of digit 2. (Cat #32.)



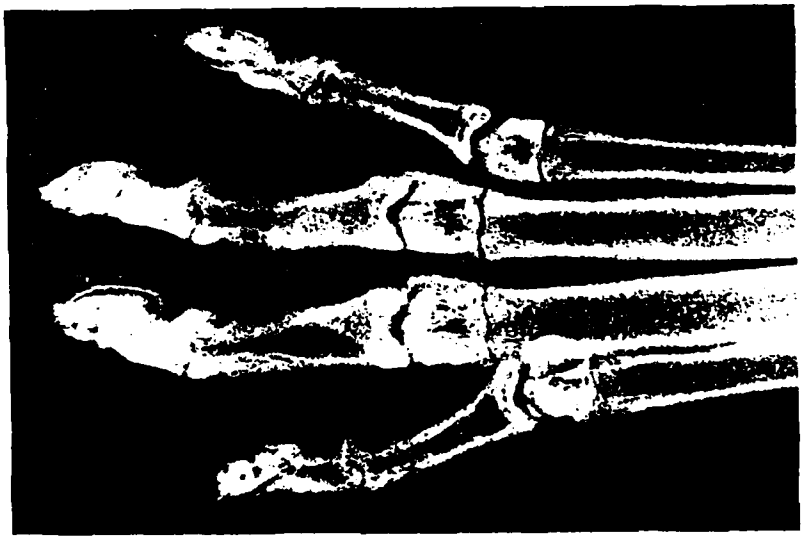
1c

Photo 2a. Radiograph, right foot, pre-injury. (Cat #24.)

Photo 2b. Radiograph, right foot, 3 months post-1st degree frostbite. "Lace-like" demineralization and loss of cortical definition of 1st and 2nd phalanges, digit 2, and 1st phalanx, digit 3. Slight cortical thinning of distal metatarsals. (Cat #24.)

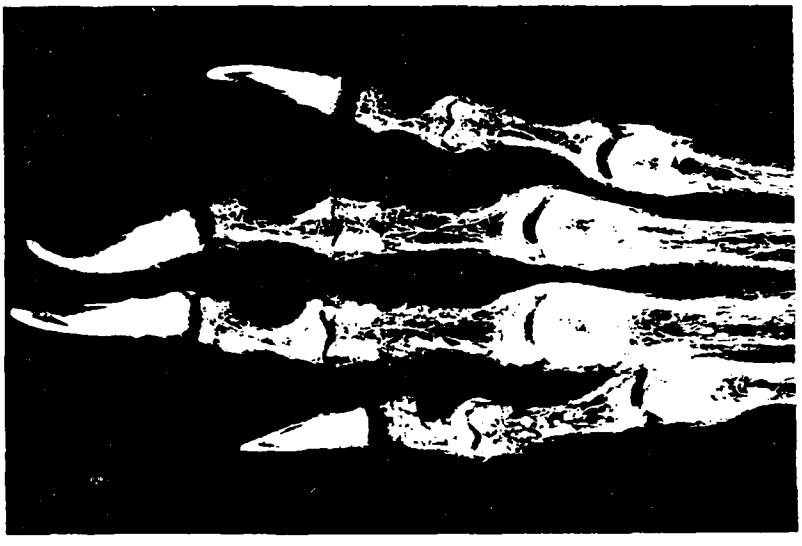


2b



2a

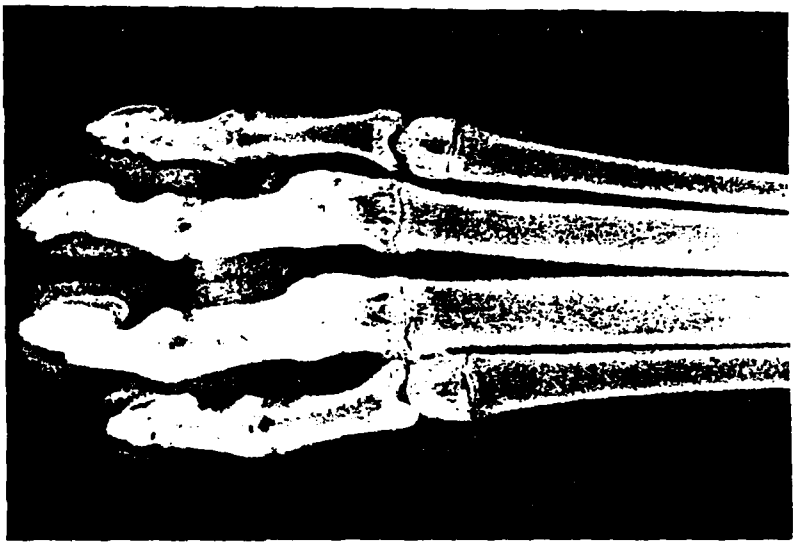
Photo 2c. Microangiograph, right foot, 3 months post-1st degree frostbite (soft tissue removed). "Pooling" of contrast media, 1st and 2nd phalanges, digits 2 and 3. Increased vascularity of distal metatarsals. (Cat #24.)



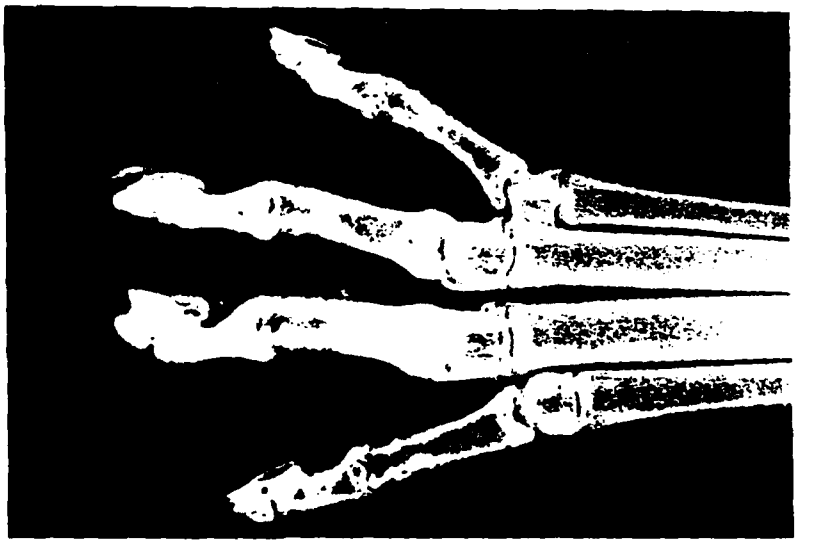
2c

Photo 3a. Radiograph, right foot, pre-injury. (Cat #9.)

Photo 3b. Radiograph, right foot, 1 month post-2nd degree frostbite. Slight generalized density loss of the distal metatarsal and 1st phalanx of digits 2 and 5. (Cat #9.)



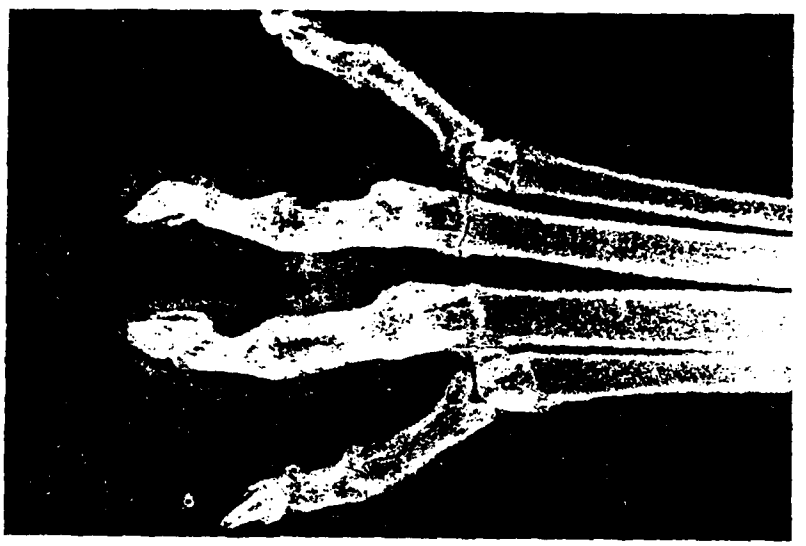
3b



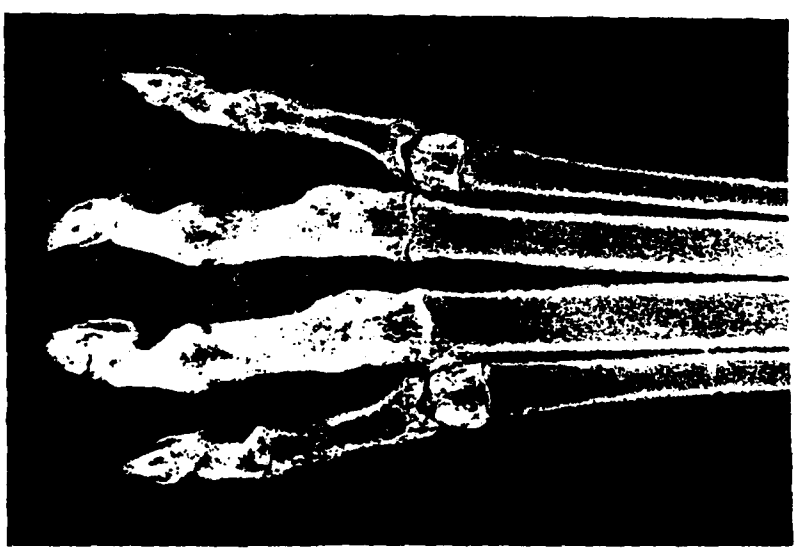
3a

Photo 3c. Radiograph, right foot, 2 months post-2nd degree frostbite. Increased radiolucency, loss of cortical definition, and trabecular accentuation in digits 2 and 5. Epiphysis of 3rd metatarsal closed. (Cat #9.)

Photo 3d. Radiograph, right foot, 3 months post-2nd degree frostbite. Diffuse loss of cortical density and trabecular accentuation of all digits. Epiphysis of 2nd metatarsal closed. (Cat #9.)

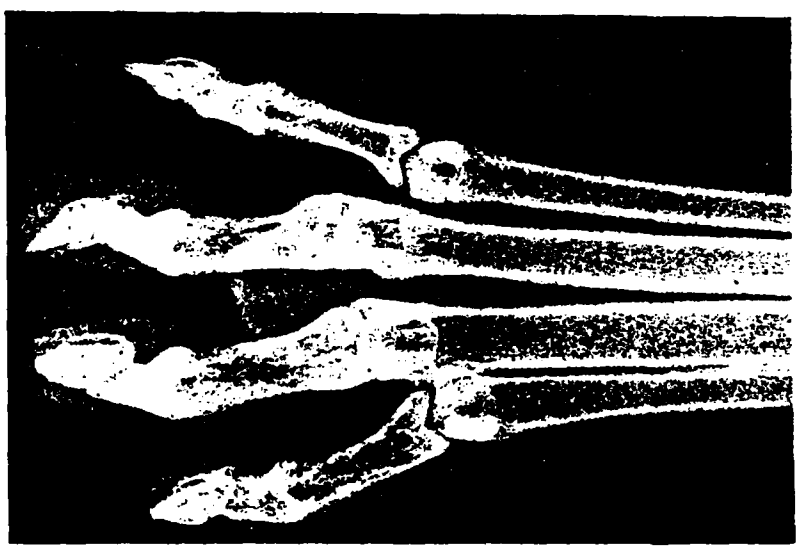


3d



3c

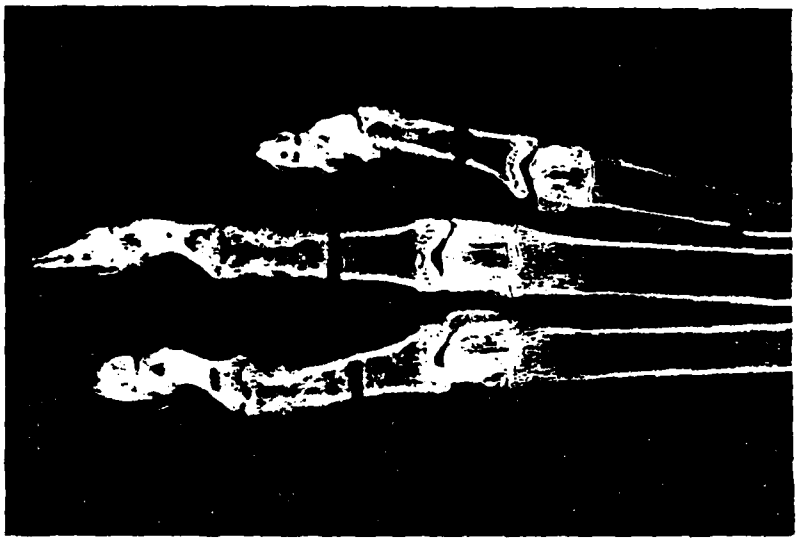
Photo 3e. Radiograph, right foot, 4 months post-2nd degree frostbite. Further cortical remodeling of 1st phalanx, digits 2 and 3. (Cat #9.)



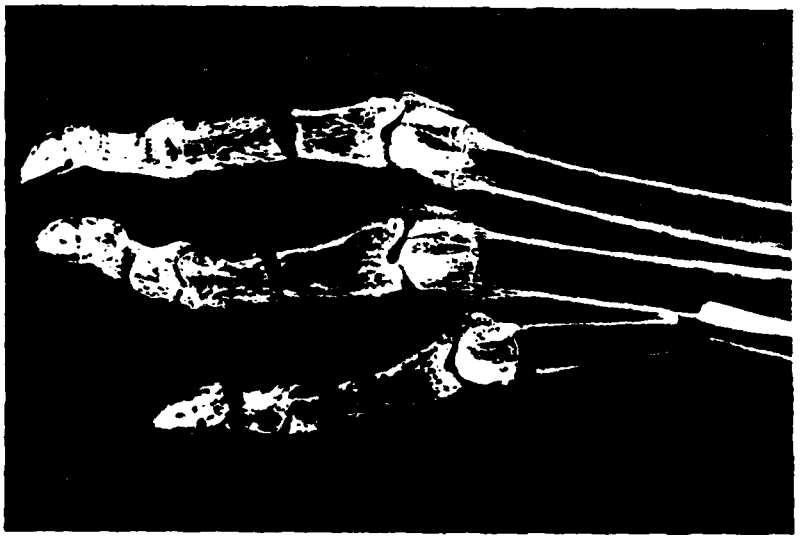
3e

Photo 3f. Soft x-ray, right foot, 4 months post-2nd degree frostbite (soft tissue removed). Increased radiolucency of metatarsals and digits, trabecular accentuation and rearrangement, joint surfaces generally spared. (Cat #9.)

Photo 3g. Soft x-ray, left foot, non-injured (soft tissue removed). (Cat #9.)



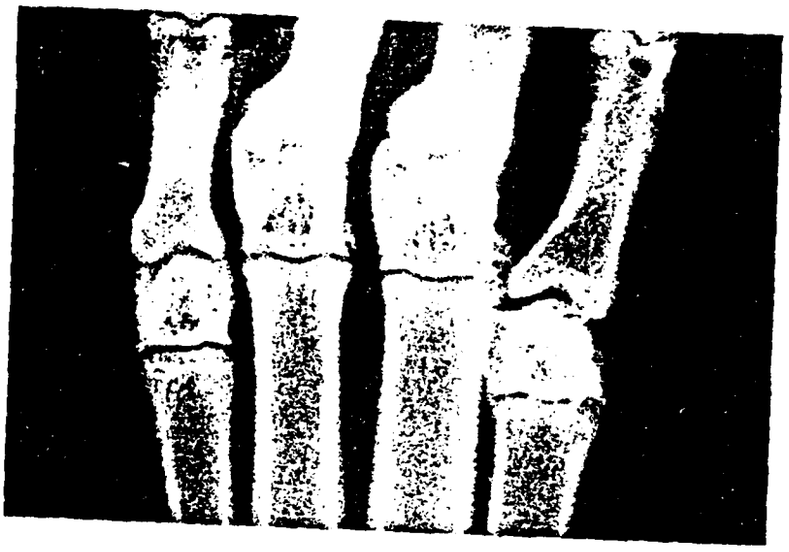
3g



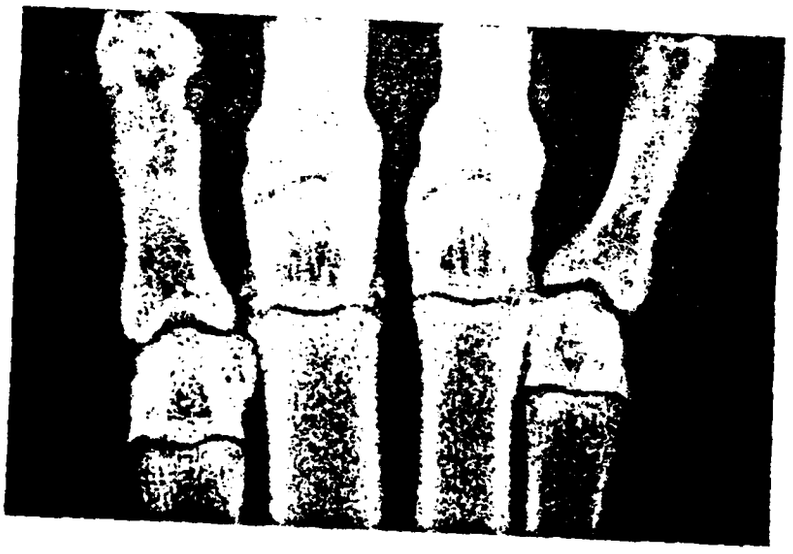
3f

Photo 4a. Radiograph, left foot, pre-injury. (Cat #27.)

Photo 4b. Radiograph, right foot, pre-injury, metatarsal epiphyseal lines open. (Cat #27.)



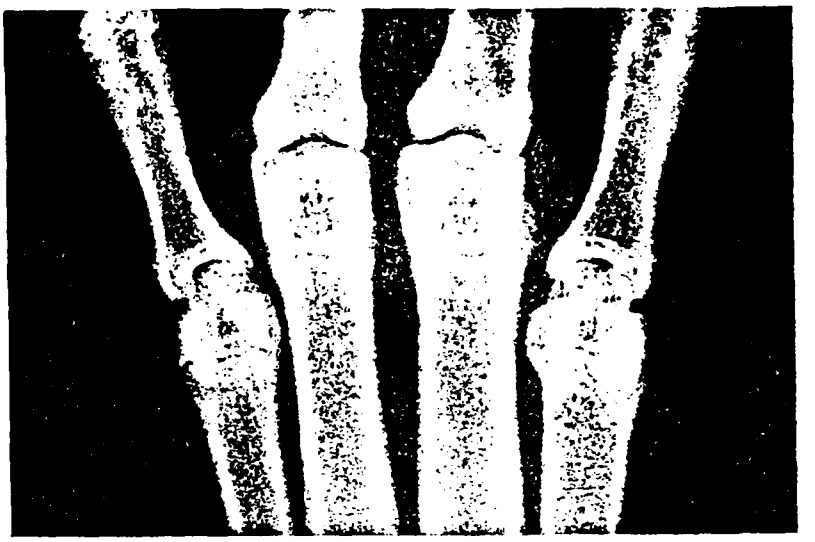
4a



4b

Photo 4c. Radiograph, left foot, 2 months, no injury. All metatarsal epiphyseal plates visible. (Cat #27.)

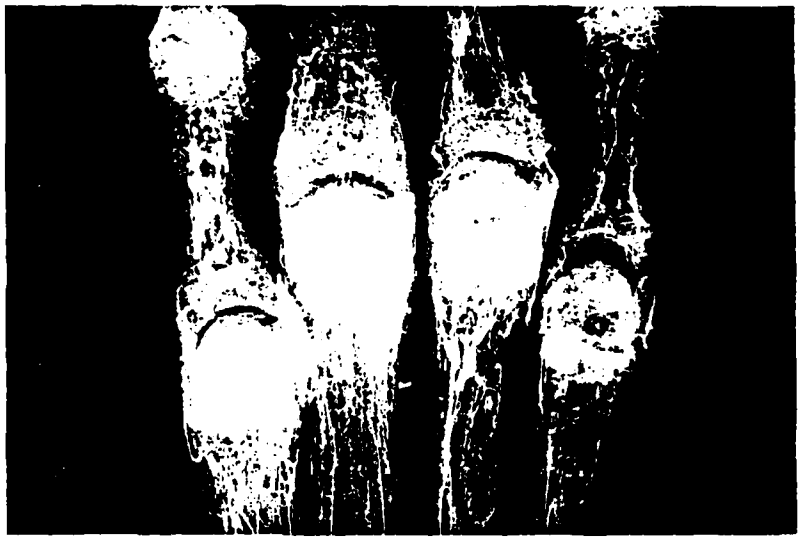
Photo 4d. Radiograph, right foot, 2 months post-3rd degree frostbite. Epiphyseal lines of metatarsals 2 and 3 closed. Diffuse radiolucency and cortical thinning of metatarsals and 2nd and 5th digits. (Cat #27.)



4c

4d

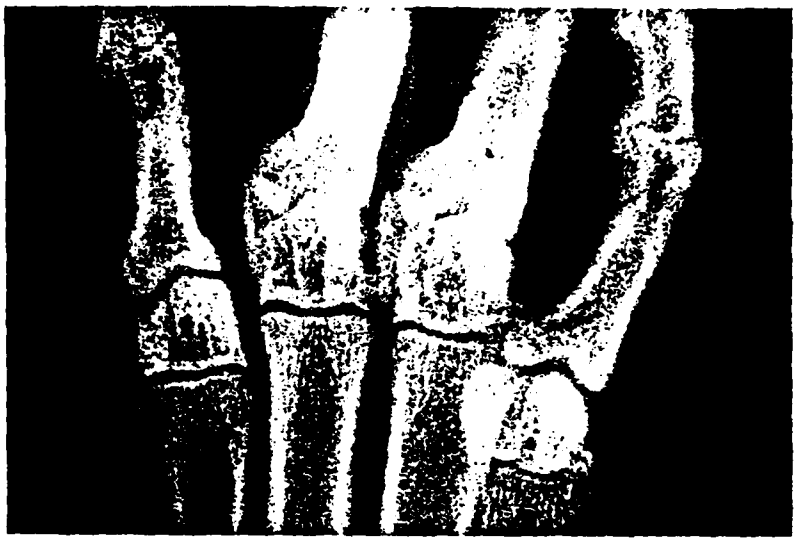
Photo 4e. Microangiograph, right foot, 2 months post-3rd degree frostbite (soft tissue removed). Apparent loss of metaphyseal blood supply to closed epiphyseal lines. General increased vascularity. Pooling of contrast media in affected epiphyses and diaphysis of last phalanx, digit 2. (Cat #27.)



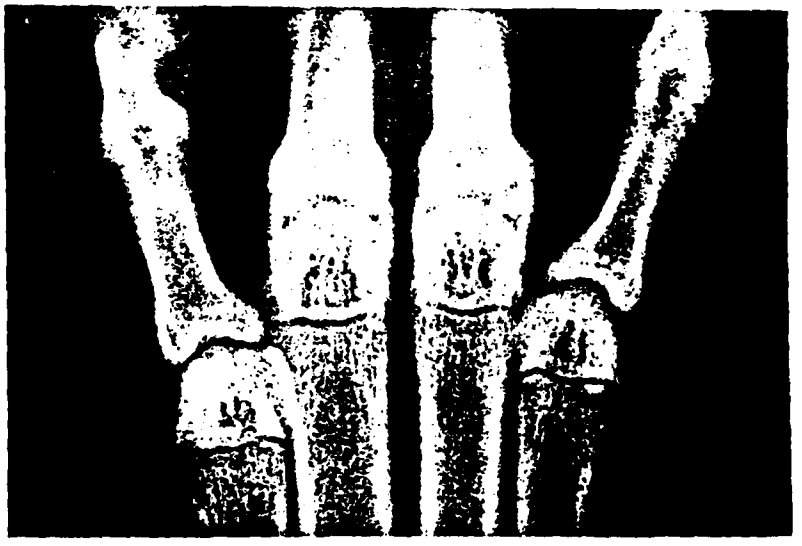
4e

Photo 5a. Radiograph, left foot, pre-injury. (Cat #10.)

Photo 5b. Radiograph, right foot, pre-injury. (Cat #10.)



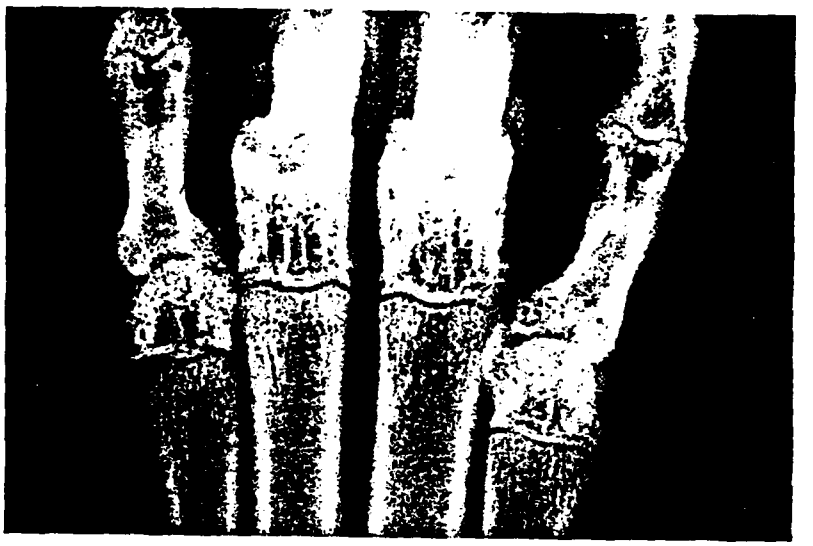
5a



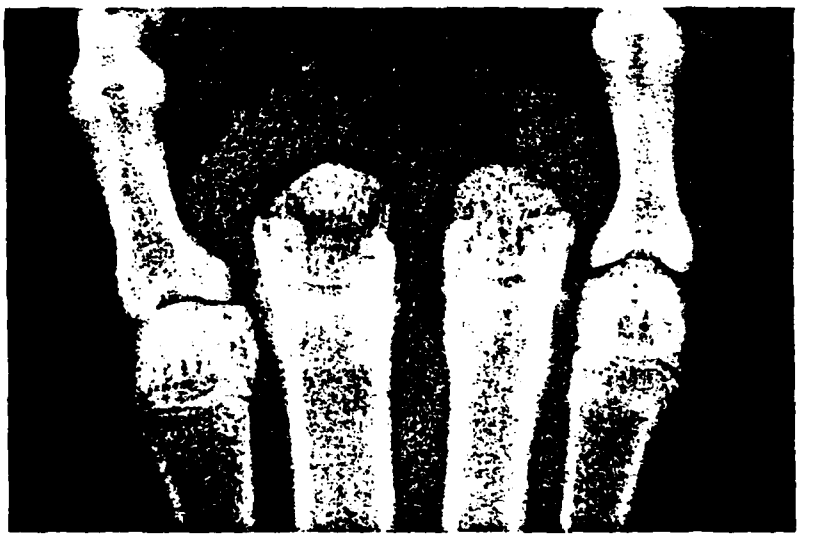
5b

Photo 5c. Radiograph, left foot, 1 month, no injury. (Cat #10.)

Photo 5d. Radiograph, right foot, 1 month post-4th degree frostbite. Entire digits 3 and 4 amputated. Distal metatarsal condyles 3 and 4 being resorbed. Metatarsal epiphyseal lines partially closed. (Cat #10.)



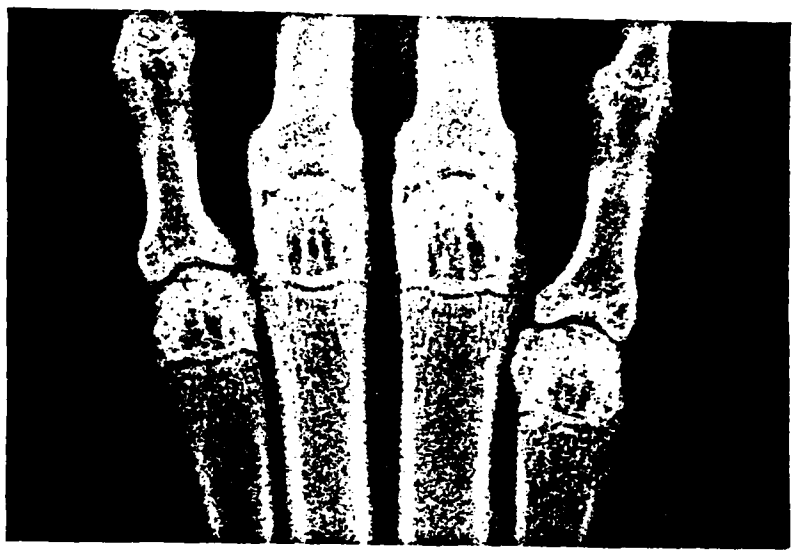
5c



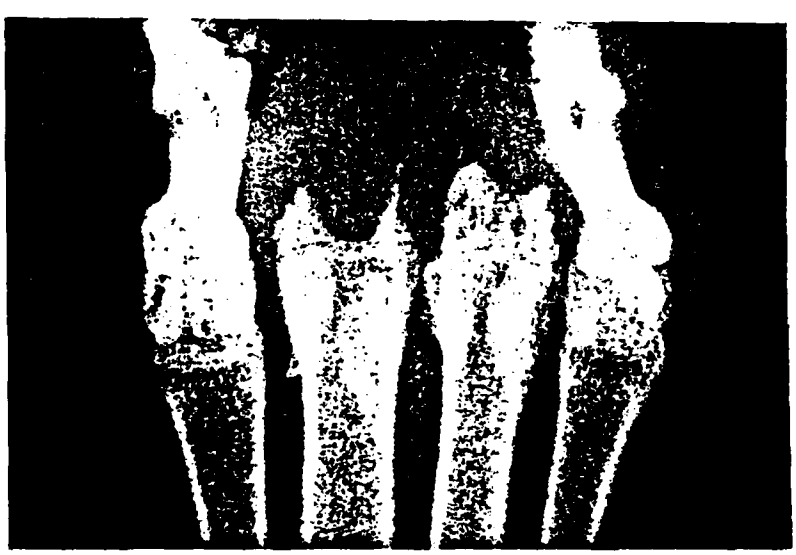
5d

Photo 5e. Radiograph, left foot, 2 months, no injury. Metatarsal epiphyseal lines open. (Cat #10.)

Photo 5f. Radiograph, right foot, 2 months post-4th degree frostbite. Further degeneration of metatarsal condyles. 3rd, 4th, 5th metatarsal epiphyseal lines closed. Apparent cortical thickening of 1st phalanx, digits 2 and 5. (Cat #10.)



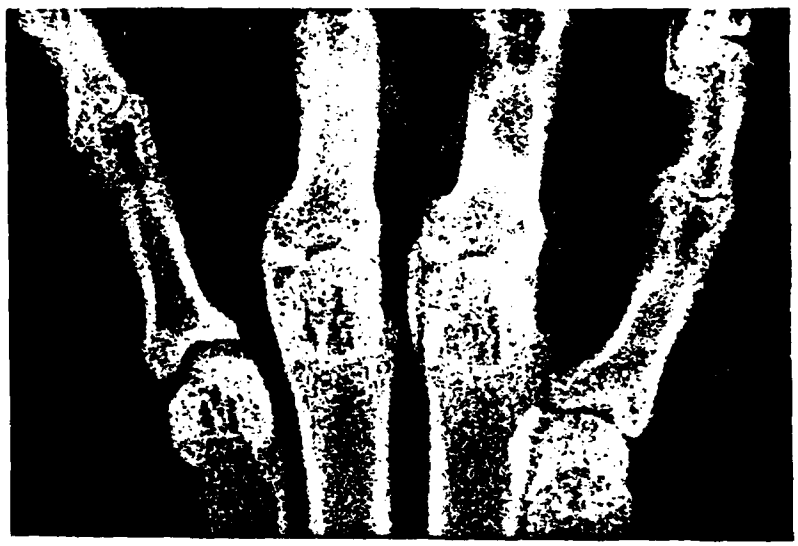
5e



5f

Photo 5g. Radiograph, left foot, 3 months, no injury. Epiphyseal lines closed. (Cat #10.)

Photo 5h. Radiograph, right foot, 3 months post-4th degree frostbite. Complete lysis of metatarsal condyles. Apparent cortical thickening of 1st and 2nd phalanges of digits 2 and 5. (Cat #10.)

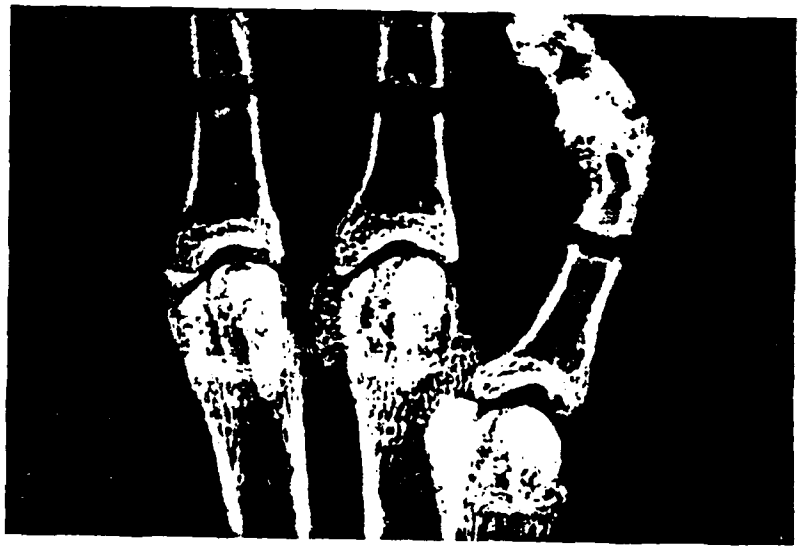


5g

5h

Photo 5i. Soft x-ray, left foot, 3 months, no injury (soft tissue removed). (Cat #10.)

Photo 5j. Soft x-ray, right foot, 3 months post-4th degree frostbite (soft tissue removed). Complete lysis of metatarsal condyles. Periosteal thickening of proximal shaft and geographic radiolucency of proximal epicondyle of 1st phalanx, digit 2. (Cat #10.)

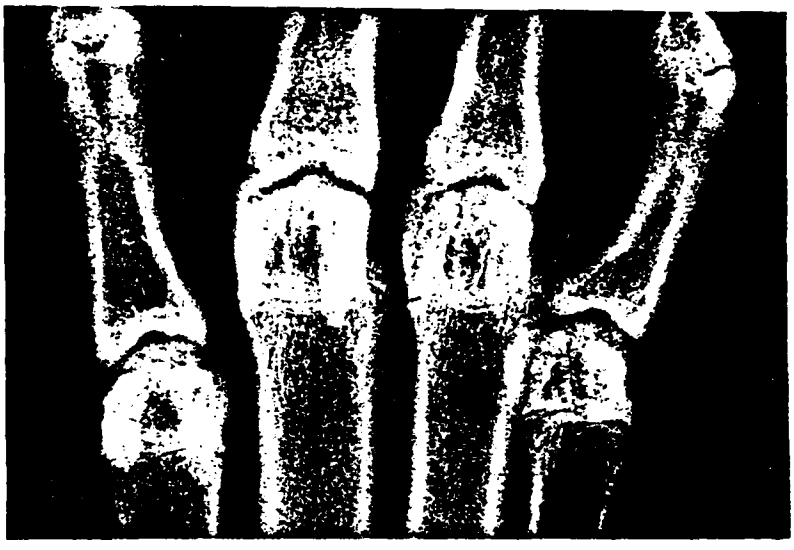


5i

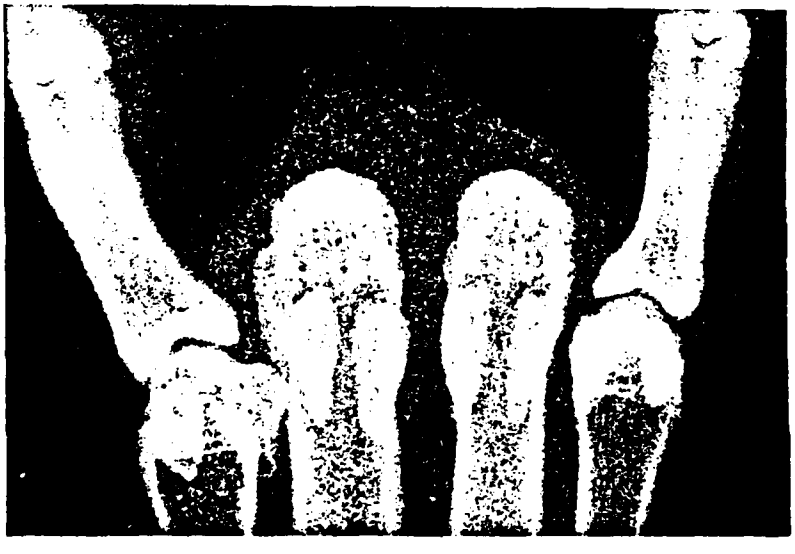
5j

Photo 6a. Radiograph, right foot, pre-injury. (Cat #30.)

Photo 6b. Radiograph, right foot, 4 months post-4th degree frostbite. Slight endosteal scalloping of 1st phalanx, digit 2 and roughening of articular surface of distal 3rd metatarsal. (Cat #30.)

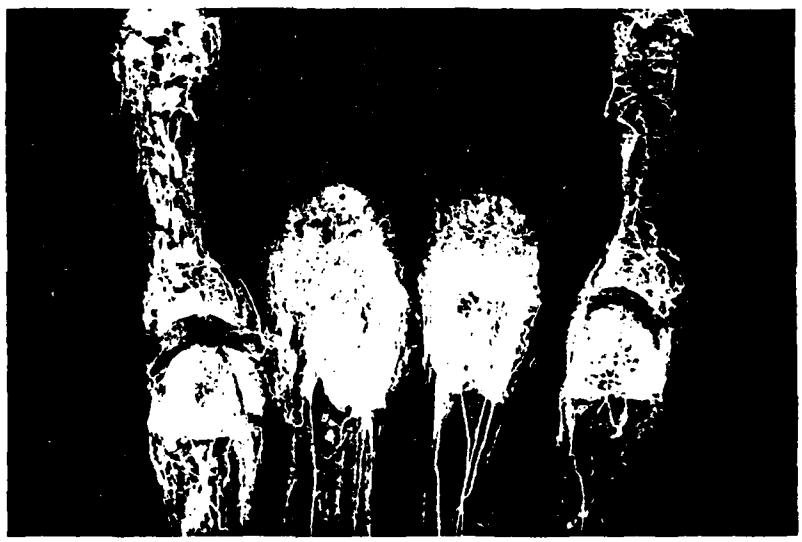


6a



6b

Photo 6c. Microangiograph, right foot, 4 months post-4th degree frostbite. Pooling of contrast media in distal 2nd metatarsal and 1st phalanx of digit 2. (Cat #30.)

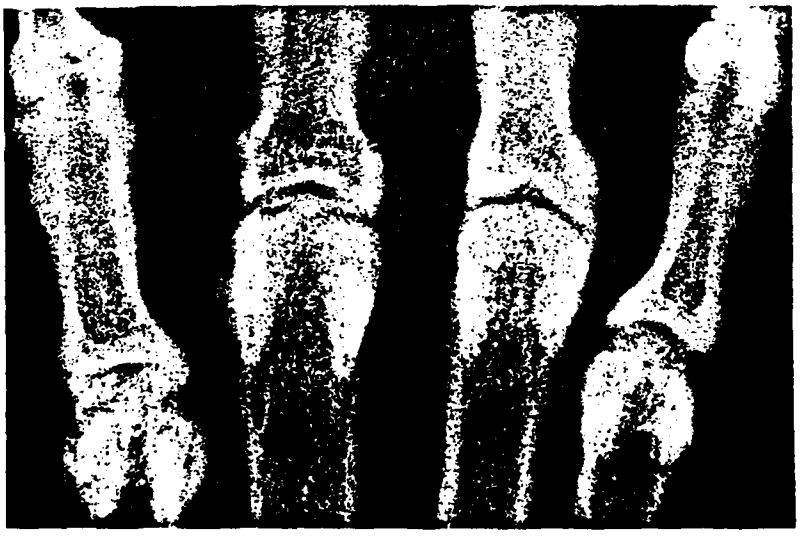


6c

Photo 7a. Radiograph, right foot, pre-injury. (Cat #14.)

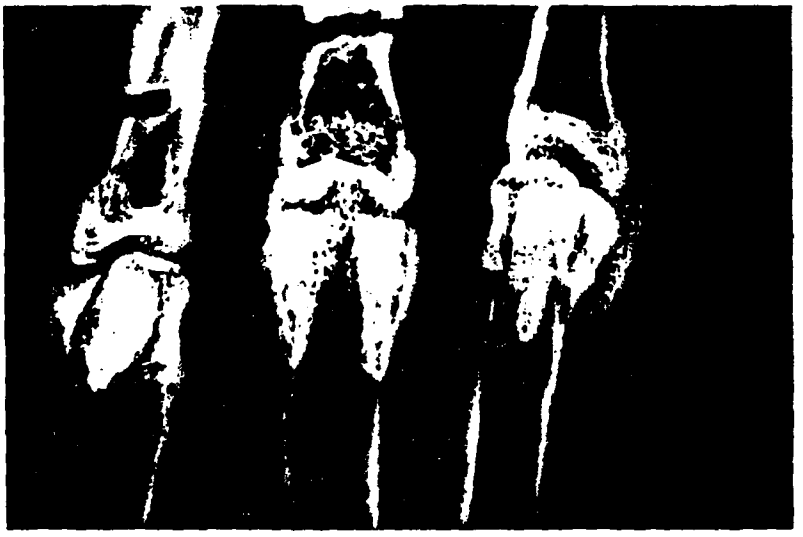
Photo 7b. Radiograph, right foot, 5 months post-4th degree frostbite. Subchondral geographic loss of density of 1st phalanx, digit 3. Ankylosis of 3rd metatarsal-phalangeal joint. (Cat #14.)

7a



7b

Photo 7c. Soft x-ray, right foot, 5 months post-4th degree frostbite (soft tissue removed). Subchondral geographic loss of density of 1st phalanx, digit 3. Trabecular rearrangement of proximal 1st phalanx, digits 2 and 3 of 3rd metatarsal phalangeal joint. (Cat #14.)



7c

Photo 8a. Radiograph, right foot, digits 3 and 4, pre-injury. (Cat #2.)

Photo 8b. Radiograph, right foot, 3 months post-4th degree frostbite. "Cyst-like" periosteal proliferation on 2nd phalanx of digits 2 and 4. Geographic sclerotic area on proximal 2nd phalanx, digit 4. Amputation of entire digit 3 and fragment of 3rd phalanx, digit 4. (Cat #2.)

8b

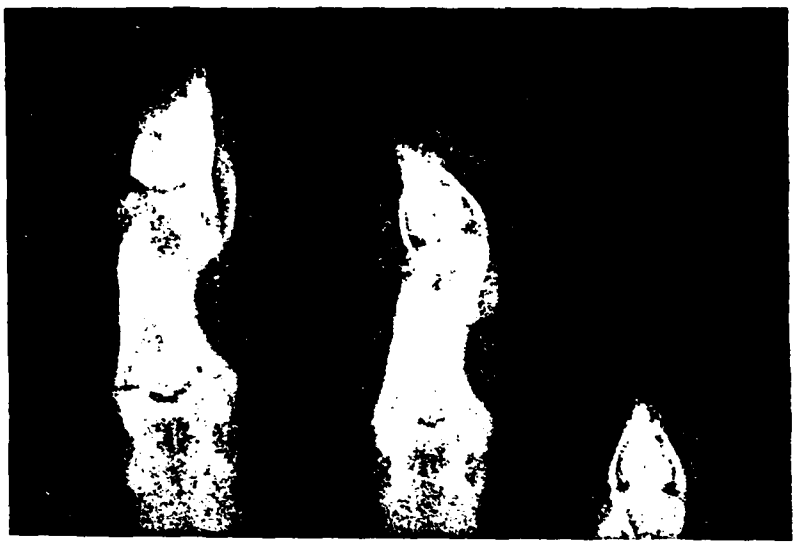
8a

Photo 8c. Microangiograph, right foot, 3 months post-4th degree frostbite (soft tissue removed). Media pooling in "cyst" on digit 2. Lack of normal vascularity of 4th digit. Increased number and tortuosity of vessels in distal 2nd metatarsal. Radiolucent areas in 4th and 5th digits are artifacts due to evaporation of ethanol and the formation of air bubbles within medullary cavities of bone specimens. (Cat #2.)

8c

Photo 9a. Radiograph, right foot, digits 3, 4, and 5, pre-injury. (Cat #31.)

Photo 9b. Radiograph, right foot, digits 3 and 4, 6 months post-4th degree frostbite. Demineralization and loss of cortical density of 1st and 2nd phalanges, digit 3. "Cyst-like" periosteal proliferation on 1st phalanx of digits 3 and 4. Partial amputation of 3rd phalanx, digit 3 and loss of toenail, digit 4. (Cat #31.)



9a

9b

Photo 9c. Microangiograph, right foot, 6 months post-4th degree frostbite (soft tissue removed). Vessel number and tortuosity increased in all digits. Vascular pooling in 2nd phalanx of digits 3 and 4. (Cat #31.)

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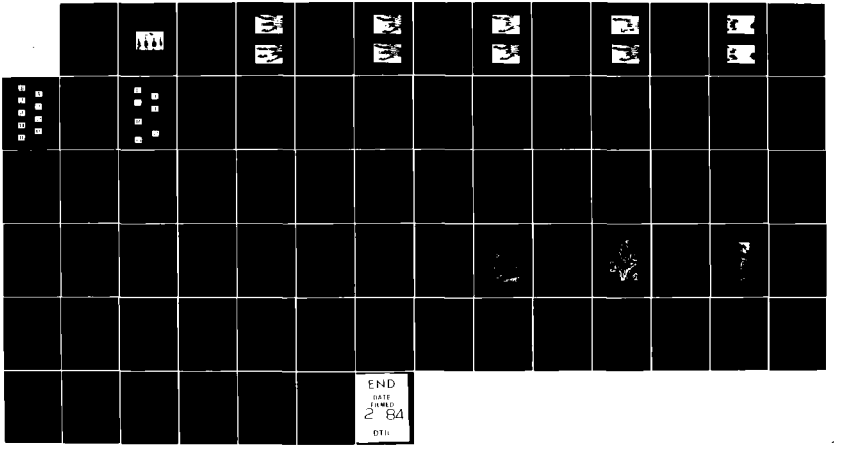

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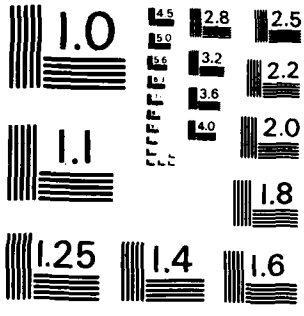
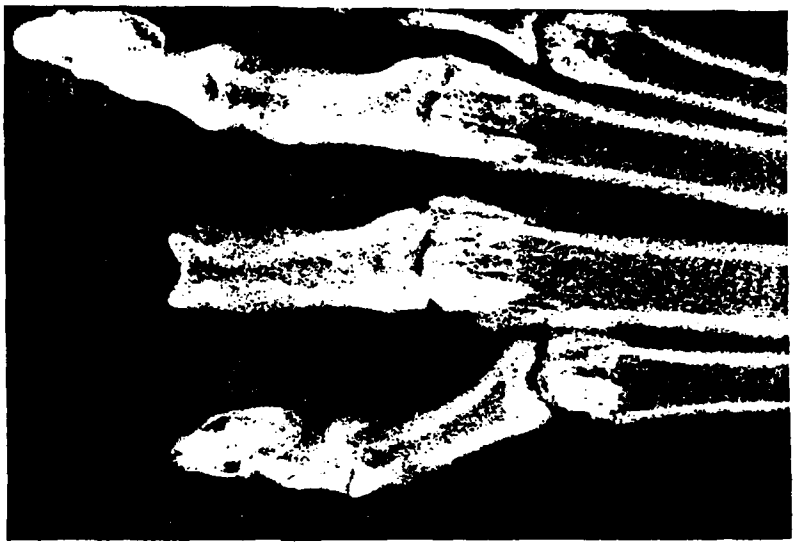


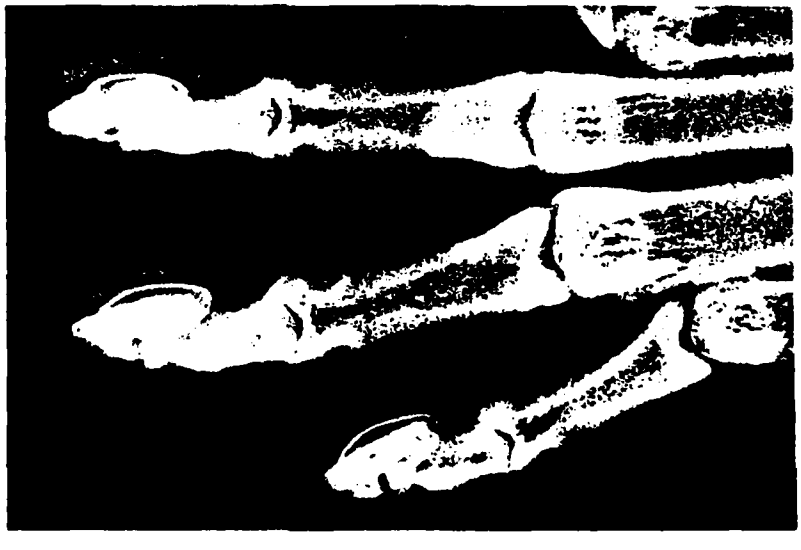
9c

Photo 10a. Radiograph, right foot, pre-injury. (Cat #13.)

Photo 10b. Radiograph, right foot, 1 month post-4th degree frostbite, 2nd and 3rd phalanges of digit 3 amputated. (Cat #13.)



10b

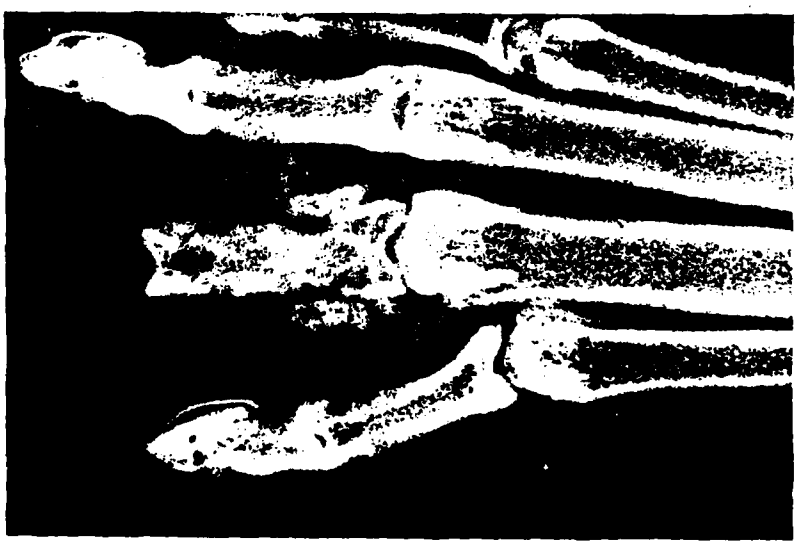


10a

Photo 10c. Radiograph, right foot, 2 months post-4th degree frostbite. Early lysis of 1st phalanx of digit 2. (Cat #13.)

Photo 10d. Radiograph, right foot, 3 months post-4th degree frostbite. Further lysis of phalanx with sparing of articular cartilage. (Cat #13.)

10d



10c

Photo 10e. Radiograph, right foot, 4 months post-4th degree frostbite. Further lysis of phalanx. (Cat #13.)

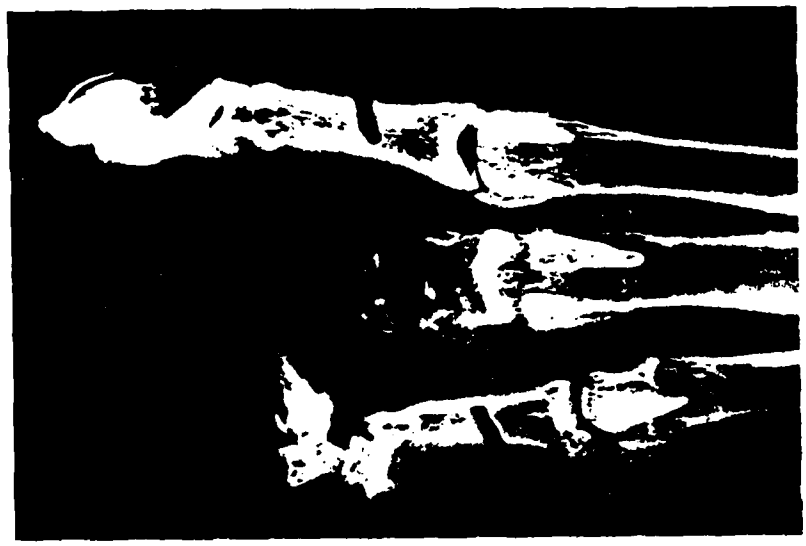
Photo 10f. Radiograph, right foot, 5 months post-4th degree frostbite. Further lysis of distal 1st phalanx. Evidence of cortical remodeling in 1st phalanx of digits 2 and 4. (Cat #13.)

10e

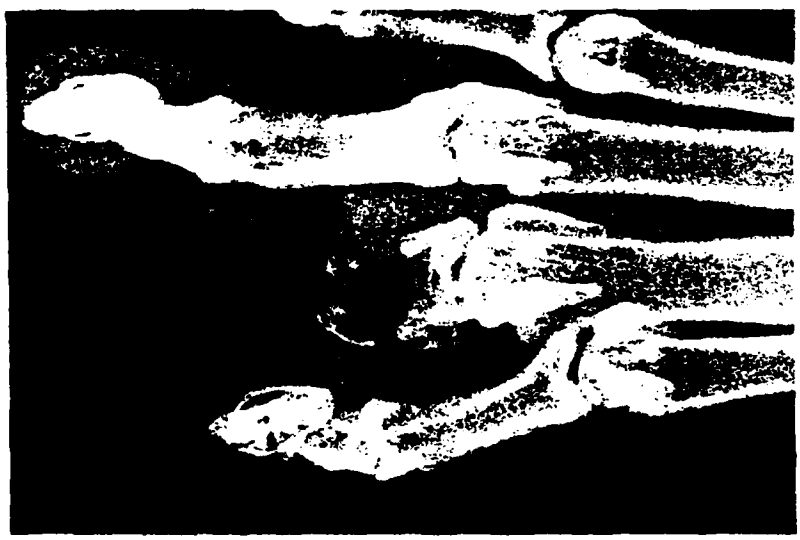
10f

Photo 10g. Radiograph, right foot, 6 months post-4th degree frostbite. Lysis of distal portion of 1st phalanx nearly complete. Metatarsal-phalangeal joint cartilage spared. (Cat #13.)

Photo 10h. Soft x-ray, right foot, 6 months post-4th degree frostbite (soft tissue removed). Lysis of 1st phalanx and ankylosis of metatarsal-phalangeal joint. Permiative resorption of 1st phalanx and apparent increased radiodensity of 2nd phalanx of digit 4. (Cat #13).



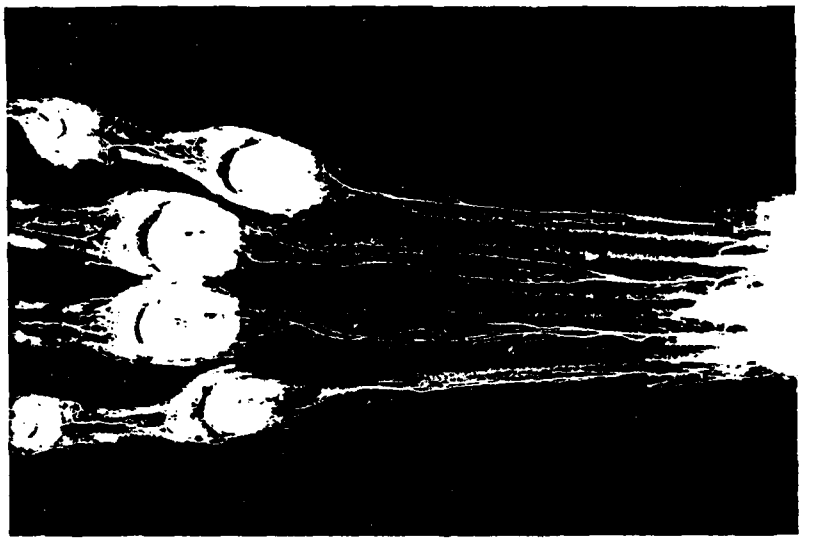
10h



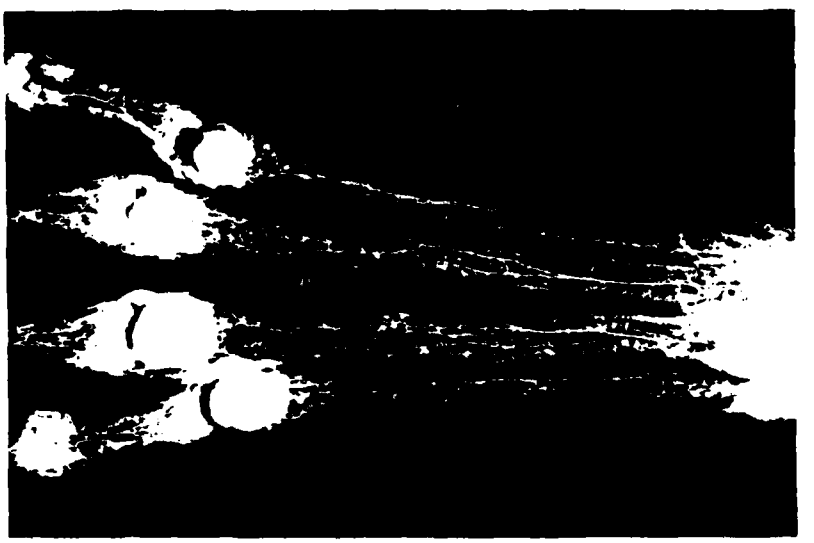
10g

Photo 11a. Microangiograph, right foot, 2 days post-3rd degree frostbite (soft tissue removed). Engorgement and micro-extravasation of metatarsal vessels. Bubble-like radiolucent areas are artifacts due to evaporation of ethanol and the formation of air bubbles within medullary cavities of bone specimen. (Cat #33.)

Photo 11b. Microangiograph, left foot, non-injured (soft tissue removed). (Cat #33.)



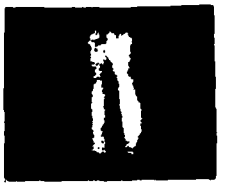
11b



11a

Photo 12. ^{99m}Tc bone scan of rear feet of cat. 4th degree frostbite resulting in amputation of 2nd and 3rd phalanges of digits 3 and 4. Lacy or mottled demineralization of 1st phalanx of digits 3 and 4 visible on radiograph at 2 months post-frostbite. Right foot on photo represents scan of right foot of cat. Bone scans were taken at pre-injury (a), 2 days (b), 2 weeks (c), 1 month (d), 2 months (e), 3 months (f), 4 months (g), 5 months (h), and 6 months (i). (Cat #19.)

a



b

c

d

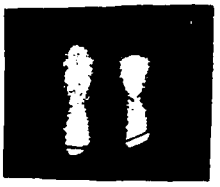
e

12

f

g

h



i

Photo 13. ^{99m}Tc bone scan of rear feet of cat. 1st degree frostbite resulting only in transient hyperemia and edema. Right foot on photo represents scan of right foot of cat. Bone scans were taken at pre-injury (a), 2 days (b), 2 weeks (c), and 1 month (d). (Cat #20.)

Photo 14. Single ^{99m}Tc bone scan of rear feet of cat. 1st degree frostbite resulting in loss of cortical definition and "lace-like" demineralization of digits. Right foot on photo represents scan of right foot of cat. Bone scan was taken at 2 months post-injury. (Cat #24.)

Photo 15. ^{99m}Tc bone scan of rear feet of cat. 2nd degree frostbite resulting in major remodeling and loss of density first visible on radiograph at 1-2 months post-injury (see photos 3a-3g). Right foot on photo represents scan of right foot of cat. Scans were taken at 2 months (a) and 4 months (b) post-injury. (Cat #9.)


a


b

13

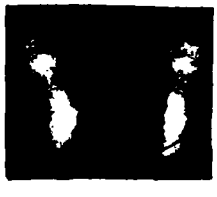

c


d

14


a

15


b

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Chapter 4

A BIOCHEMICAL EVALUATION OF COLD-INDUCED BONE LESIONS IN THE CAT

Few references are made to the problem of skeletal changes following acute or chronic exposure of the extremities of man to the cold (1-9). The lesions have been described radiographically and classified as (1) osteoporosis, (2) acromutilation, (3) juxta-articular areas of decreased density, and (4) other bone changes such as periostitis, new bone formation, and osteomyelitis (5). The most common radiographic abnormality, osteoporosis, often is seen proximal, but not distal, to the area of injury, serving as an indicator of intact or altered circulation and metabolism. This radiographic lesion closely resembles that seen in disuse atrophy or microcirculatory disturbance in bone, which results in negative calcium balance (10). Specific laboratory findings in cold-induced bone lesions seldom have been reported or have been absent (1); cold agglutinins, cryoglobulins, etc. have not been demonstrated. Sedimentation rates have been normal and rheumatoid factors have been absent (1). Biochemical, immunological, and bone ash studies in animal models have not been reported. The objective of this study was to produce bone lesions in the hind limb of the domestic cat by subjecting it

to mild frostbite injury, then to examine the animals biochemically and immunologically, and to evaluate bone samples from the animals by ashing.

MATERIALS AND METHODS

Frostbite injury was produced in eight male and nine female domestic cats by exposing one hind foot to -50° C moving air until temperature deep within the foot reached approximately -10° C. The method of injury production and post-injury care has been described previously (chapter 2). All animals were maintained on free-choice cat chow and water in standard cages. Seven were maintained for six months post-injury while the other ten were sacrificed in pairs at 2 days, 2 weeks, 1, 2, and 4 months post-injury. Data were collected and evaluated as described in Table 1.

Blood samples were collected from the jugular vein between 9 and 12 a.m. Animals were anesthetized with Ketamine HCl (20 mg/kg, IM) when necessary to avoid struggling. Blood for erythrocyte sedimentation rates was mixed immediately with 3.7% sodium citrate and analyzed by the modified Westergren method within 1 hour of collection. Samples for WBC and platelet count were transferred immediately to appropriate Unopett^{R} diluting solutions and analyzed within 2 hours. Blood for serum studies was clotted and the serum was collected in siliconized glass tubes and analyzed by a clinical veterinary laboratory within 12-18 hours after the blood was drawn. Twenty-four hour urine samples were collected using a method that has been described previously (Appendix 1). Urine

Table 1. Samples collected, time of collection, and method of analysis.

At pre-injury and 2 days, 2 weeks, 1, 2, 3, 4, 5, and 6 months post-injury:

Plasma serum:	Calcium (colormetric) (11) Inorganic phosphorus (colormetric) (11) Total protein (colormetric) (12) Alkaline phosphatase (colormetric) (13) Antinuclear antibodies (indirect immunofluorescence microscopy) (14) Feline rheumatoid factor (standard Waller Rose method, agglutination) (15)
Whole blood:	WBC Microhematocrit Platelet count (phase contrast microscopy/Unopett^{R}) Erythrocyte sedimentation rate (Westergren method) (16)
Urine (24 hour):	Calcium (as above) Inorganic phosphorus (as above)

At pre-injury and monthly post-injury:

Hind feet:	High contrast radiographs for densitometry

At time of sacrifice:

Bone:	Intramedullary pH (micro combination glass pH probe) Fifth metatarsal bone from injured and noninjured limbs for microincineration Final radiograph for densitometry

was analyzed for calcium and inorganic phosphorus by the clinical laboratory within 12-18 hours after collection. Radiographs of injured and non-injured limbs were made on high contrast, non-screen industrial film. The final film taken on the day of sacrifice was evaluated using a digital densitometer.

Intramedullary pH was measured in several animals under pentobarbital anesthesia just prior to euthanasia. An incision was made over the dorsal aspect of the mid shaft of the 2nd metatarsal and the shaft was transected using electrician's wire cutters. The tip of a 1.2 mm glass combination pH microelectrode was placed quickly within the medullary cavity of the proximal fragment. pH readings were recorded at 30 seconds after insertion of the electrode. Following euthanasia, the fifth metatarsal of each injured and non-injured limb was removed and cleaned of soft tissue. The epiphyses were separated from the shafts, and the marrow was cleaned from the medullary cavities. The bones were cleaned of fat, dried at 110° C, and a fat-free dry-weight was obtained. Bones then were incinerated in a muffle furnace at 550° C for 16 hours and the ash was weighed.

Data from biochemical and densitometry studies were collected from the animals at prescribed intervals of time after injury; the data for each time period were related because the same animals were represented at each point. Grouped data were first analyzed by the Friedman two-way analysis of variance and Wilcoxin matched pairs signed-ranks

test. To overcome the problem of related data, the values for each individual animal were fitted to a regression line, and the attitude of the resulting slope (i.e., positive or negative) was evaluated using the non-parametric Sign test. In retrospect, when plotted data indicated definite trends, slopes of portions of the data were evaluated using the Sign test. Means and standard errors are plotted, and the results of the evaluation of the individual slopes are described in the figure legends. Ashed bone data were analyzed by the Paired t test (18, 19).

RESULTS

a. Injury produced

Two animals had local hyperemia and edema (1st °) following injury, one animal showed vesicle formation (2nd °), one lost only soft tissue (3rd °), and five lost both soft tissue and bone from one or more phalanges (4th °). No correlation was seen between degree of injury and other parameters measured.

b. Mineral metabolism

Total calcium levels measured in serum were corrected for differences in serum protein (Figure 1) (20), and Ca^{++} levels were calculated (Figure 2) by the method of McLean and Hastings (21). Neither total calcium nor Ca^{++} levels were significantly altered from pre-injury levels throughout the post-injury evaluation period. Serum inorganic phosphorus remained statistically within the pre-injury limits through-

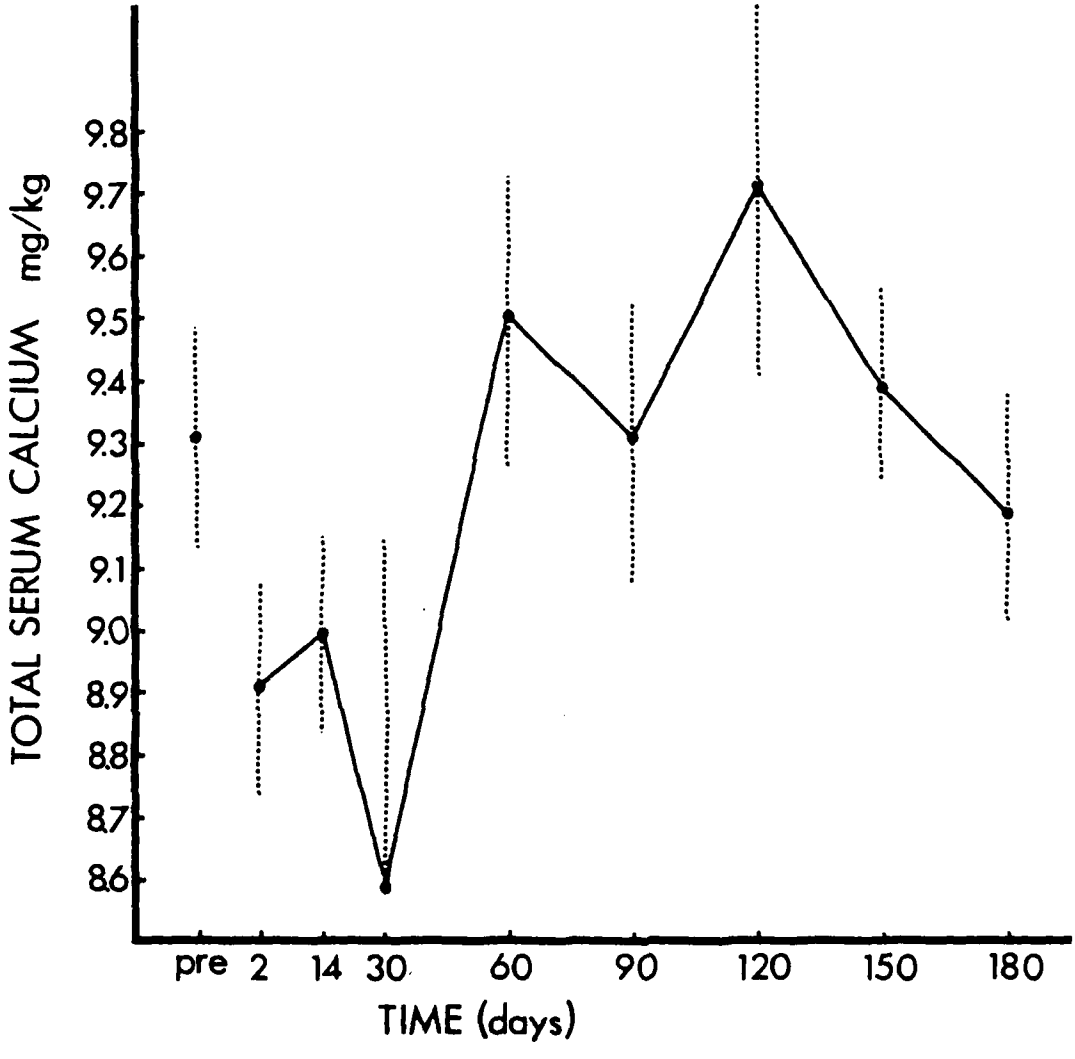

Figure 1. Total serum calcium corrected to a standard 6.6 gm % protein. Correction is based on the assumption that calcium changes by 0.054 gm % for every 0.1 gm % change in total protein. Both analysis of variance and individual regression analysis indicated that post-injury levels did not differ significantly from pre-injury levels. Bars indicate ±1 SEM. (Pre-120 days, n = 9; 150-180 days, n = 7.)

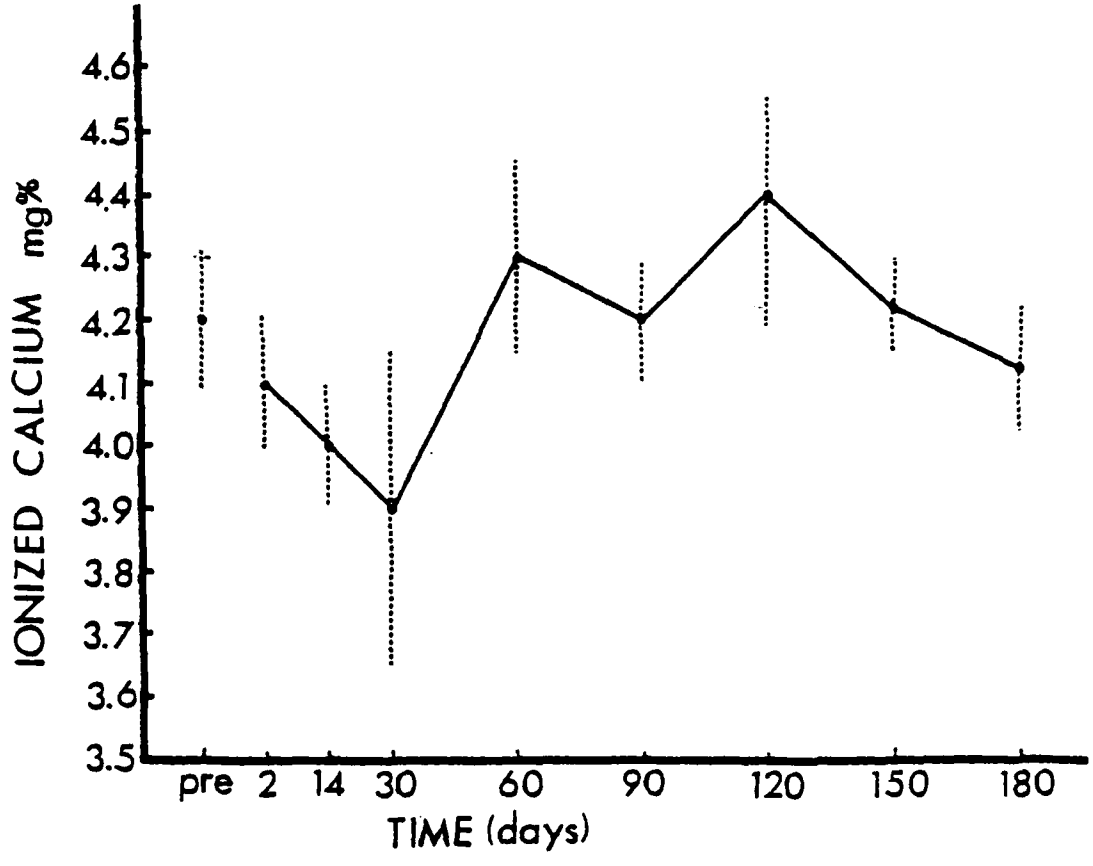

Figure 2. Mean concentration of ionized calcium in the serum calculated from total serum calcium and plasma protein. Levels did not deviate significantly from pre-injury throughout the study. Bars indicate ±1 SEM. (Pre-120 days, n = 9; 150-180 days, n = 7.)

out the study (Figure 3). Urine calcium and phosphorus were significantly different from pre-injury levels only at three to six months post-injury, when both were lower than pre-injury levels. Urine calcium (Figure 4) levels generally dropped between 2 and 6 months post-injury. Urine phosphorus levels dropped continually ($P = 0.02$) throughout the study (Figure 5). Serum alkaline phosphatase (Figure 6) levels rose significantly during the first 4 months post-injury and remained within normal limits after that time.

c. Immunology

For the first 60 days post-injury, the mean erythrocyte sedimentation rates fell significantly ($P = 0.02$). Sedimentation rates stabilized well below pre-injury levels at 60 days and for the rest of the study (Figure 7). Positive titers to feline rheumatoid factor and antinuclear antibodies, although low (1:4-1:6), were seen in several animals, usually immediately after injury, as indicated by Table 2. Positive results were noted twice for the same test in only two animals; one showed a positive feline rheumatoid factor on the pre-injury test and the two week test, and the other showed successive positive tests for feline rheumatoid factor at 2 days and 2 weeks post-injury.

d. Microincineration

The fat-free dry weight of the injured 5th metatarsal bones of 16 animals was significantly less ($P < 0.01$) than that of their contralateral controls. Mean percent ash of non-injured (68.8 ± 0.5) and injured (68.6 ± 0.6) metatarsals was not significantly different ($P = 0.34$).

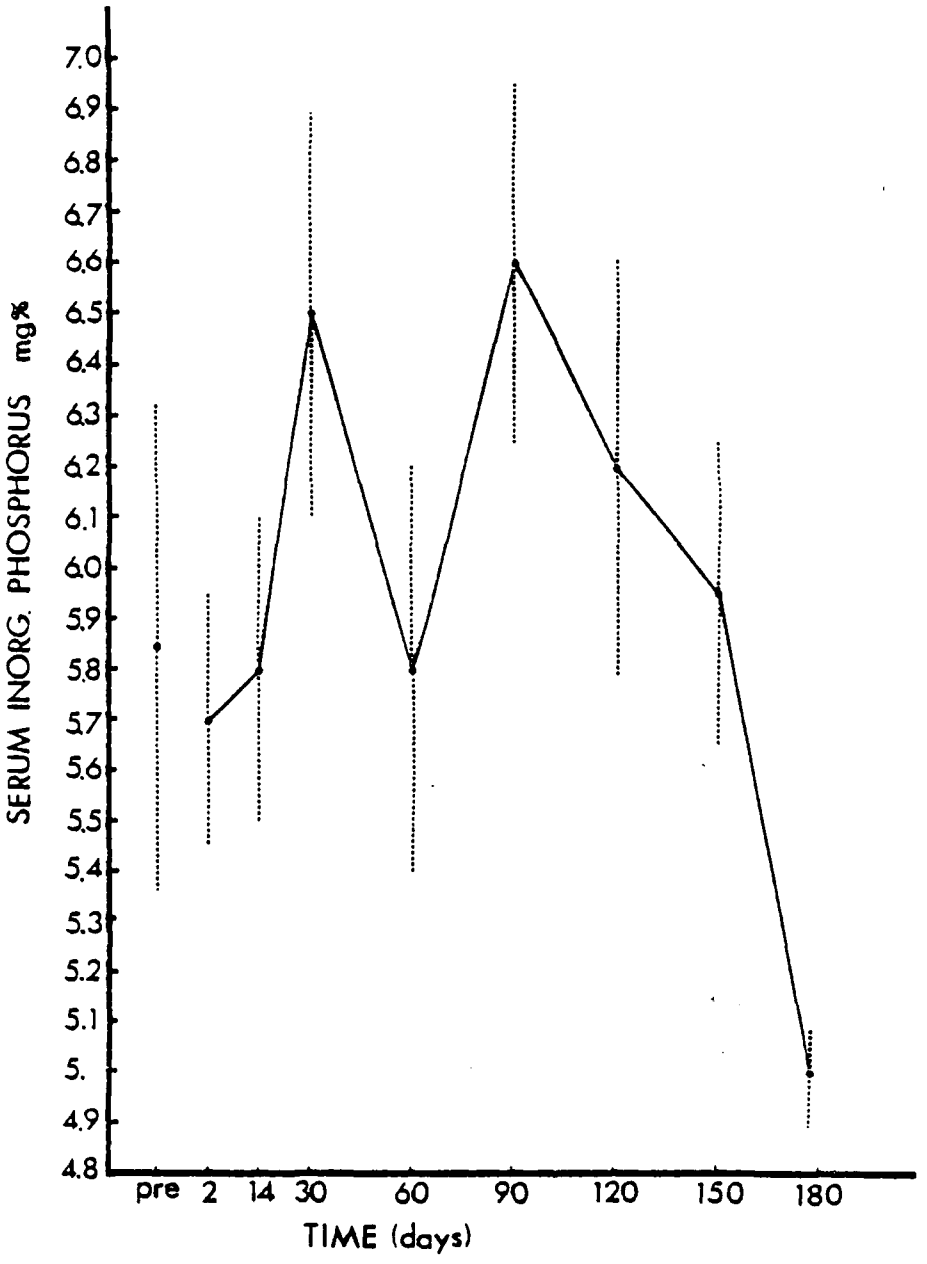

Figure 3. Concentration of inorganic phosphate in the serum. Values did not deviate significantly from pre-injury levels during the post-injury period; however, levels appeared to be elevated slightly between 30-150 days and depressed at 180 days post-injury. Bars indicate ±1 SEM. (Pre-120 days, n = 9; 150-180 days, n = 7.)

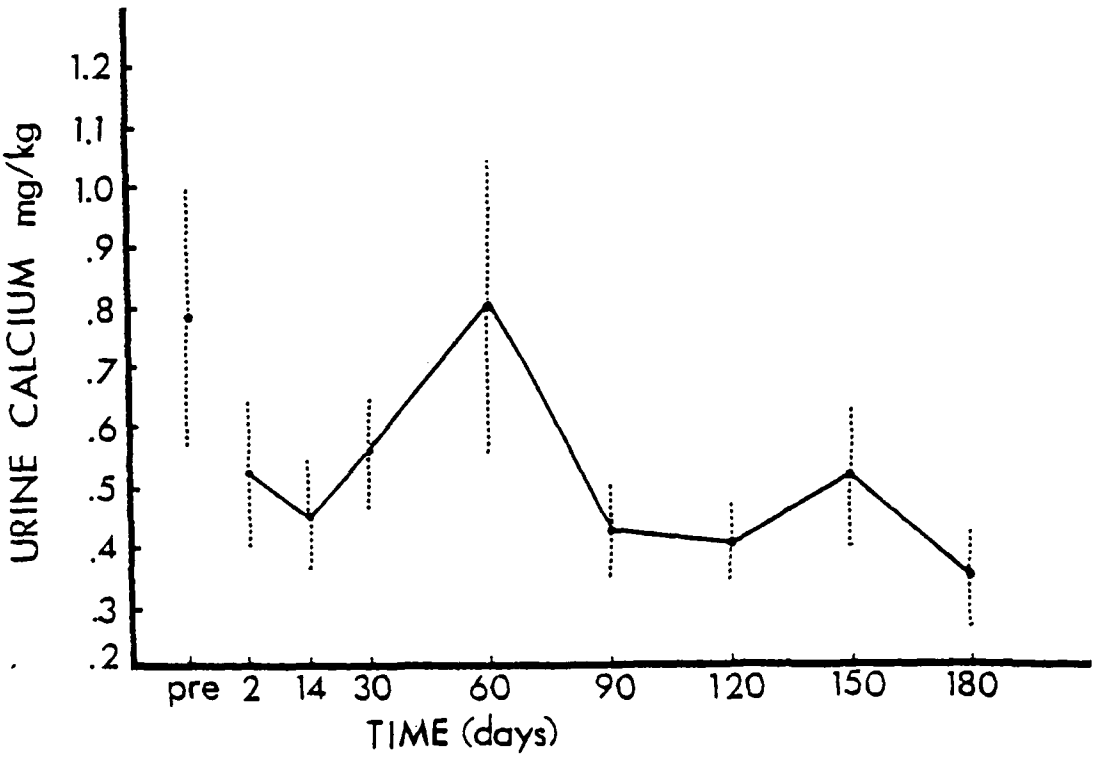

Figure 4. Urine calcium (mg/kg/day) calculated from 24 hour urine samples. Evaluation of regression lines representing the data of individual animals showed a general decrease in urine calcium levels during the period from 60 to 180 days post-injury ($P = 0.02$). Bars indicate ±1 SEM. (Pre-120 days, $n = 9$; 150-180 days, $n = 7$.)

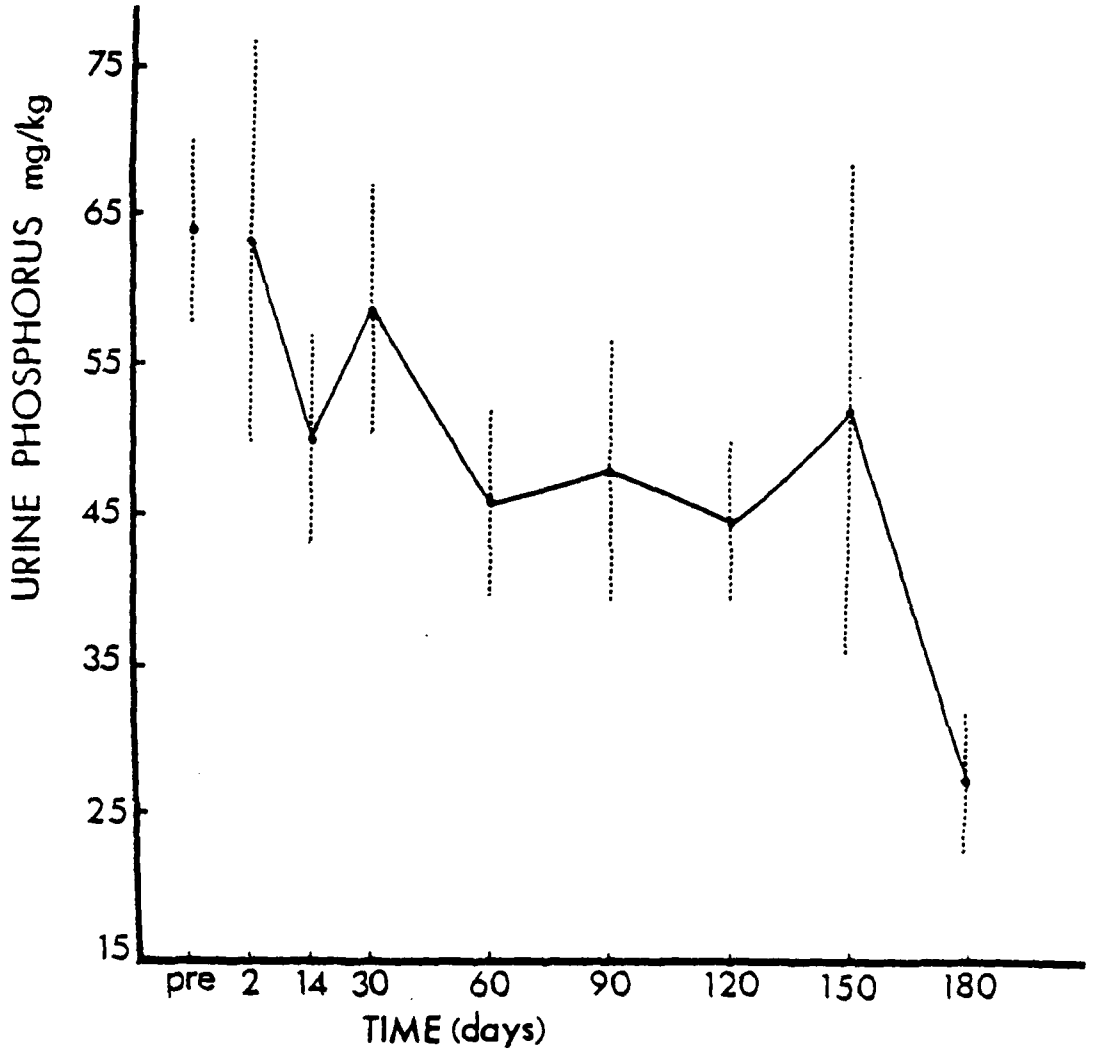

Figure 5. Urine phosphorus calculated from 24 hour urine samples. Regression lines representing the data of individual animals from before to 180 days after injury showed a decline in urine phosphorus throughout that period ($P = 0.02$). Bars indicate ±1 SEM. (Pre-120 days, $n = 9$; 150-180 days, $n = 7$.)

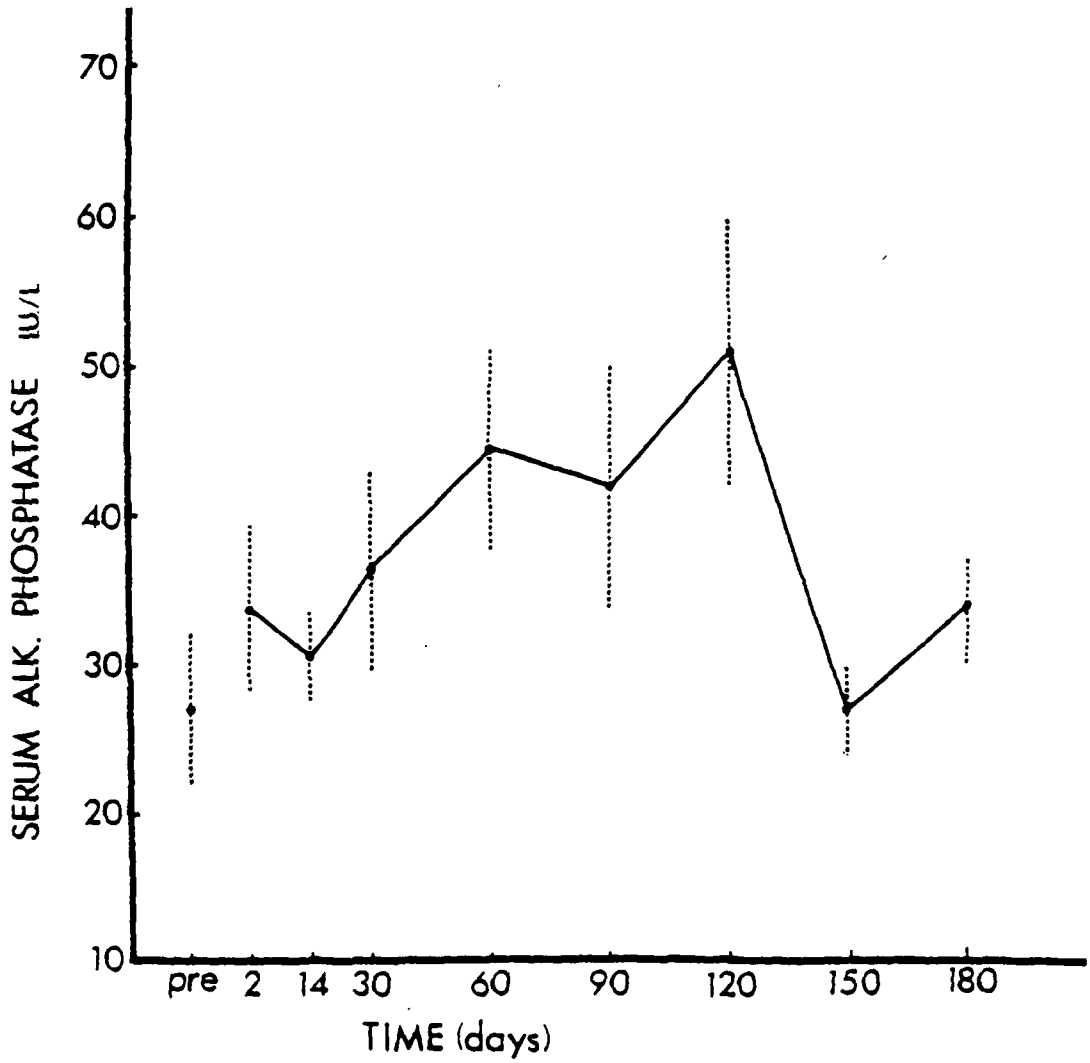

Figure 6. Serum alkaline phosphatase. Regression lines representing data from individual animals showed a general rise in serum alkaline phosphatase from before to 120 days after injury ($P = 0.002$). 60 day measurements were significantly elevated over pre-injury readings ($P < 0.01$). Bars indicate ±1 SEM. (Pre-120 days, $n = 9$; 150-180 days, $n = 7$.)

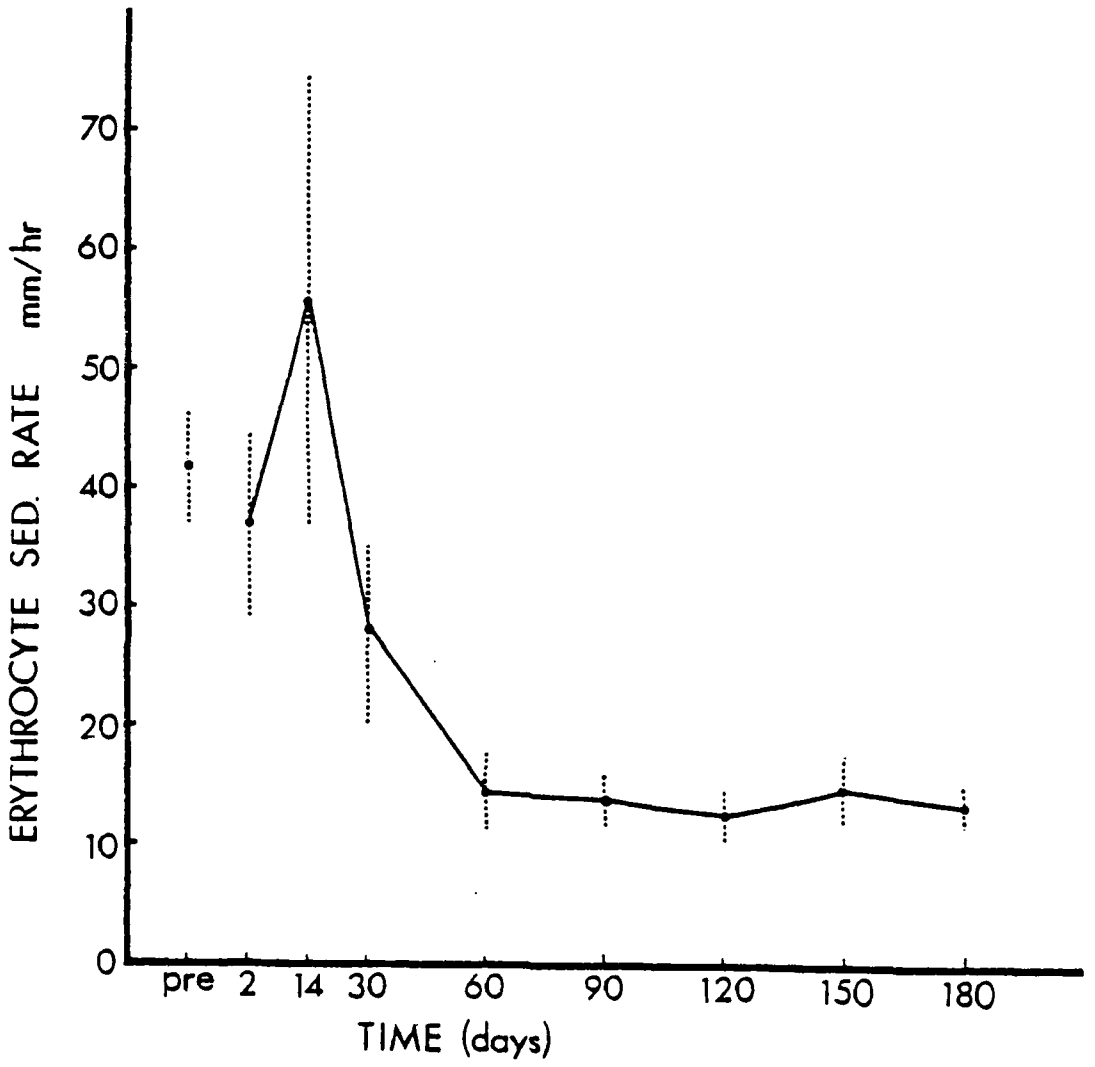

Figure 7. Erythrocyte sedimentation rates. A general decline in erythrocyte sedimentation rate was seen from before to 60 days after injury ($P = 0.02$). The sedimentation rate stabilized within normal limits between 60 and 180 days post-injury ($P = 0.25$). 60 day rates were significantly below pre-injury levels ($P < 0.01$) and remained low through 180 days post-injury. Bars indicate ±1 SEM. (Pre-120 days, $n = 9$; 150-180 days, $n = 7$.)

Table 2. Positive titers to feline rheumatoid factor (FRF) and antinuclear antibodies (ANA).

Time of Analysis	FRF Positives	ANA Positives
Pre-injury	1 of 17	0 of 17
2 days	3 of 17	0 of 17
2 weeks	2 of 17	4 of 17
1 month	0 of 15	1 of 15
2 months	1 of 13	2 of 13
3 months	0 of 11	0 of 11
4 months	1 of 11	0 of 11
5 months	0 of 9	0 of 9
6 months	0 of 9	0 of 9

e. Radiograph densitometry

Optical density was measured in all metatarsals of 9 animals at three points within a 2 cm area of the metatarsal midshafts. Density was significantly reduced in all injured metatarsals on final radiographs ($P \leq 0.05$) when compared to the non-injured foot. Individually, only one animal failed to demonstrate density loss in the fifth metatarsal; the same animal also failed to show loss of fat-free dry weight in the injured fifth metatarsal. Densities of injured and non-injured fifth metatarsal shafts were compared throughout the period of study. Figure 8 is a plot of the mean log ratios of injured/non-injured radiographic bone density measured before injury and monthly thereafter. A value of zero on this graph indicates equality, while a negative value indicates that the injured bone was less radiodense than its non-injured counterpart. The log ratios were evaluated by the relationship between the slopes of the values of individual animals. There was a significant trend toward decreased radiodensity throughout the period of study ($P = 0.02$).

f. Medullary pH

The pH of the midshaft medullary cavities of the injured 2nd metatarsal bones was not significantly different from the non-injured contralateral controls in animals tested. The pH of injured medullary cavities measured 7.28 ± 0.04 and non-injured cavities measured 7.31 ± 0.04 ($P = 0.64$).

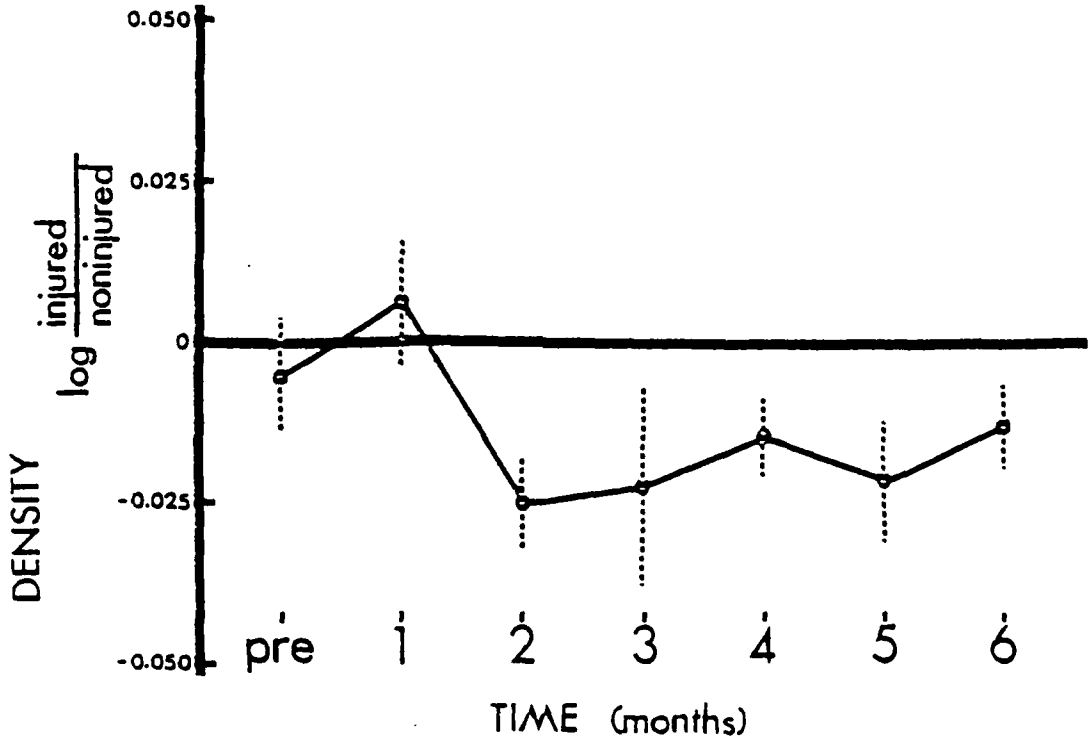

Figure 8. Radiodensity of metatarsals of injured vs non-injured feet. Data based on densitometry reading taken from 3 points along midshaft of 5th metatarsal and plotted as mean log injured density/noninjured density. Bars indicate ±1 SEM. (Pre-4 months, n = 9; 5-6 months, n = 7.)

DISCUSSION

Frostbite occurs when heat is transferred from living tissue to the surrounding environment to the extent that ice crystals form in the tissue. Because the heat gradient depends primarily on factors external to the body, frostbite is by nature a graded injury. The most severe injury usually is found in the most distal portion of the extremity. Less severe grades of injury are represented more proximally along the limb. With this principle in mind, one may evaluate the results of the present study.

Injury was produced in this study by cooling the limb until a given area of tissue, deep within the foot near the distal metatarsals, reached a temperature of approximately -10° C. Apparently because of individual variation in vascular response to cold, the phalanges received varying degrees of cold injury under this protocol, while the metatarsal region received a first degree (hyperemia and edema) injury in all animals. Therefore, biochemical and immunological data were based on a group of animals that have various degrees of cold injury, while microincineration and medullary pH data are from a group of metatarsal bones that sustained a relatively similar mild frostbite injury.

The reduction in fat-free dry weight without a change in percent ash content is by definition osteoporosis, a condition in which bone of normal composition is reduced in amount (22). Most types of osteoporosis in man result from increased resorption of endochondral bone at a rate greater than periosteal or haversion system bone is being

laid down (23).

Plasma calcium levels reported here are calculated Ca^{++} and total Ca corrected for plasma protein. These values are thought to approximate true Ca^{++} (24) which, though difficult to measure, is the physiologically active plasma fraction. Ionized calcium is the form that relates to parathyroid hormone secretion, for example. Calcium and inorganic phosphorus in the plasma typically are normal in osteoporosis, although both may be elevated in severe osteoporosis, due to rapid resorption of skeletal mineral (25). Plasma calcium and inorganic phosphorus did not change after injury; these findings are compatible with the presence of an osteoporotic process.

Urine calcium and phosphorus never were elevated significantly, and were reduced during the final three months of this study. Evaluating calcium or phosphorus balance in the body by measuring urine calcium is somewhat like watching only one exit of a hotel in an effort to understand how much linen is used in the guest rooms. Absorption of calcium and phosphorus in the gut is linked and depends on active metabolites of vitamin D and parathyroid hormone. The mineral source for these subjects was free-choice dry cat food. The skeleton is by far the most important repository of calcium in the body and also accounts for a major fraction of body phosphorus. In normal adult man, 15 to 20% of the calcium that is excreted is found in the urine, and most of the remaining 80-85% is excreted in the feces (26). Considering the complexity of the body's calcium and phosphate homeostasis, urinary output can

serve, at best, as a crude indicator of bone turnover, and then only if dietary intake, fecal loss, and renal function are assumed to be stable. If these assumptions are made, the data indicate a general positive calcium and phosphorus balance during the period of 3 to 6 months post-injury. During this period, loss of calcium and phosphorus in the urine was less than normal.

Serum alkaline phosphatase was elevated during the first 2-4 months post-injury. Skeletal alkaline phosphatase is located within osteoblasts and possibly osteocytes (25). In the absence of liver disease, this indicates bone turnover (27). It is not known whether alkaline phosphatase reflects bone formation or resorption, although most evidence supports formation (28). The common feature of bone diseases in which serum alkaline phosphatase is high is the presence of increased osteoblastic activity with or without associated bone destruction. Purely destructive bone lesions probably do not give rise to elevated serum alkaline phosphatase (29). The alkaline phosphatase levels recorded in the present study suggest increased osteoblastic activity during a period of 2-4 months post-injury.

The erythrocyte sedimentation rate fell dramatically during the first two months post-injury, after an insignificant elevation at two weeks post-injury. The normal sedimentation rate for the domestic cat is 7 to 23 mm/hr. Sedimentation rate is a nonspecific test useful in diagnosing and treating many diseases. It is increased in the rheumatoid family of diseases, apparently because the concentration of plasma

proteins is abnormal. A normal erythrocyte sedimentation rate is not seen in the presence of clinically active rheumatism, provided the patient is untreated (30). Erythrocyte sedimentation rate is elevated in local inflammatory conditions of the skeleton, apparently as a result of increased fibrinogen levels. The local inflammatory reaction following cold injury may explain the sedimentation rate elevation during the first 30 days post-injury, and the low normal values from 2 to 6 months post-injury suggest that autoimmune disease is not a part of the skeletal response to local cold injury. Failure of cold-injured animals to demonstrate significant titers to antinuclear antibodies or feline rheumatoid factor further strengthens the case against autoimmune involvement. Feline rheumatoid factor titers normally exceed 1:40 in 80% of cats that have rheumatoid arthritis (31).

Serial radiographic densitometry indicated that loss of bone substance from the metatarsals began at 2 months post-injury and may have been preceded by a period of decreased bone loss relative to the contralateral uninjured limb.

Frost (23) describes remodeling of bone as the turnover of bone in microscopic packets. A histological unit of cells involved in remodeling is coordinated in such a way that formative activity normally follows resorptive activity in a given area. The physiological consequence of this "basic multicellular unit" is the maintenance of skeletal tissue, replacing tiny damaged regions of bone in endosteal, periosteal, and haversion envelopes. Initiating stimuli, working times,

and numbers of cells involved in these units are controlled by local and extrinsic factors both physiologically and pathologically. A stimulus "activates" mesenchymal precursor cells at the bone surface, and the cells proliferate over a period of days. Part of the precursor cells differentiate into osteoclasts that resorb a packet of bone during a period of about 1 month, then osteoblasts appear in the same area and lay down new bone during the next several months. Time periods may vary with type of initiating stimulus and nature of local biochemical environment, between species and even within species (23, 32). Spatially, the basic multicellular units move across the surface of the bone in the envelope in which the process was initiated. An external stimulus such as a crush, contusion, burn, acute denervation, or injury to the main artery that supplies a part is known to activate simultaneously a supraphysiological number of basic multicellular units. In this way, a mass resorptive/formative cycle is begun. The osteoporosis that results has been described as "post-traumatic osteodistrophy," a reversible phenomenon that can occur without disuse of the extremity involved. It is reversible in the sense that when the bone heals, the osteoporosis also heals, although abnormal trabecular patterns can be seen for months or years (23). This syndrome has not been described following cold injury.

The findings reported here are compatible with the concept of the basic multicellular unit, and more specifically, synchronous activation of such a process by an acute stimulus. The osteoporotic lesions

described by Vinson (5) following cold injury in man also fit the general "basic multicellular unit" scheme. Vinson saw osteoporosis at 4 to 10 weeks post-injury but seldom after 6 months post-injury. Figure 9 adds Frost's basic multicellular unit scheme to a summary of the data from this study. The initial cold assault triggers the process which, after the first cycle, becomes asynchronous and may continue beyond the predicted 4 month period because of either spatial movement of basic multicellular activity along a bony surface, or continued secondary stimuli. The 2-6 week delay in activation and the prolonged activation of basic multicellular unit activity might be explained if a secondary factor such as an increase in vascularity (Chapter 3) were a primary stimulus required for activation of basic multicellular units.

SUMMARY

One hind foot of each of 17 cats was exposed to cold air to produce local cold injury. Blood, urine, and bone samples were collected at monthly intervals for up to 6 months post-injury. Plasma calcium and phosphorus remained unchanged, although alkaline phosphatase was elevated from 2 to 4 months post-injury. Urine calcium and phosphorus were decreased from 4 to 6 months post-injury. Bone ash data indicate a true osteoporosis beginning at 2 months post-injury. Sedimentation rates, feline rheumatoid factor, and antinuclear antibody findings suggest that immunological phenomena were not involved in the post-frostbite bone changes. It is proposed that exposure to low temperature stimulates

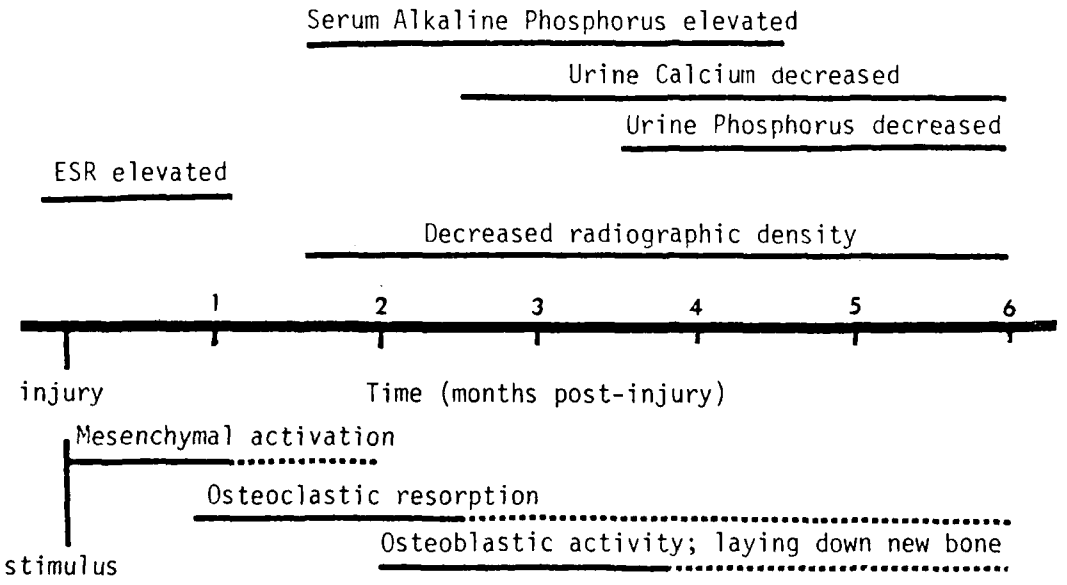

Figure 9. Above the time line: Summary of major bone-related biochemical findings following frostbite. Below the time line: Summary of previously proposed "basic multicellular unit" scheme.

synchronous activation of supraphysiologic numbers of histological bone remodeling units, resulting in bone loss.

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Chapter 5

HISTOLOGIC EVALUATION OF COLD-INDUCED BONE LESION IN THE CAT

The radiographic appearance of cold-induced bone lesions in man and experimental animals has been described (Chapter 3). Histopathological studies of these lesions have been limited (1, 2). Amputation following frostbite injury usually is performed at 3-16 weeks post-injury and involves primarily the removal of avascular bone and soft tissue (3). The bone changes seen after cold injury occur in perfused bone. For this reason, adequate bone biopsies are rarely available for histopathologic examination. Kulka examined bone from the frostbitten feet of rabbits described radiologically by Schatzki (4, 5). He reported lytic necrosis of subperiosteal osteocytes (3 days post-injury), periosteal proliferation and revascularization followed by resorption of underlying dead cortex (6 days), and dense periosteal new bone growth (16 days). The purpose of the present study was to examine histologically skeletal tissue from the feet of cats following frostbite injury. The method of injury and the radiographic and biochemical studies of the lesions have been described previously (Chapters 2, 3, and 4).

MATERIALS AND METHODS

Soft tissue was removed from the feet at the time of sacrifice. Reference radiographs were made using a soft x-ray unit. The cortex of each metatarsal and phalangeal bone was cut at two sites with a Stryker^{R} saw to allow adequate penetration of the fixing solution. Bones were placed in 10% formalin. At the completion of the study, 3-4 small samples of bone (approximately 5 x 5 x 5 mm) were cut from each foot for histological examination. All samples were decalcified in formic acid (6) and stained with hematoxylin and eosin. Slides of 96 bone samples from injured and non-injured feet were evaluated single blind by a pathologist (7).

RESULTS

There was no microscopic evidence of injury to bone in the feet of two animals sacrificed 2 days after frostbite injury. Although injuries were graded as 3rd and 4th degree, nuclei were visible within lacunae of cortical distal metatarsal bone, and articular cartilage of first interphalangeal joints appeared normal. At 2 weeks post-injury, severe subperiosteal resorption was seen in a foot that sustained 3rd degree injury, and purulent granulation tissue was visible adjacent to bone in a 4th degree injury. Diffuse loss of density was first noted by soft x-ray at 2 weeks post-injury. Samples taken at one month through six months post-injury generally depicted various stages of resorption and reconstruction of bone. Peak remodeling of bone occurred

between 2 weeks and 2 months post-injury. Osteoclastic resorption of bone was seen on periosteal and trabecular surfaces, and fibrous infiltration occurred in trabecular spaces. Increased vascularity was noted on periosteal and joint capsule surfaces (Photos 1-3). Joint cartilage was almost universally intact and appeared viable, although reconstructive activity was seen in nearby subchondral areas of bone. Osteocytes usually were nucleated, and bone appeared to have been viable adjacent to the areas of severe osteoclastic resorption at the time of sacrifice. Non-injured limbs showed either no significant lesion or small local resorptive areas thought to indicate normal maintenance activity.

Massive subperiosteal resorption and proliferation of the osteogenic layer of periosteum was the most striking feature of the histological lesions seen in injured bone. When resorption was evident, it was always present on the subperiosteal surface, although not always on the endosteal or trabecular surfaces. Resorption was seen in areas that showed radiographic lesions and in areas that showed no significant lesions.

DISCUSSION

Bone dynamics in health and disease normally involve remodeling by resorption and formation, which occur sequentially in a given area. Resorption occurs on the three anatomically and physiologically distinct surfaces found within bone, i.e., endosteal, haversion, and periosteal envelopes. On endosteal surfaces, resorption usually exceeds formation;

Photo 1. Photomicrograph (135x) of the shaft of a metatarsal bone 1 month post-injury. Note thickened periosteum (P) with new vascularity (NV). Several osteoclasts (OC) are present in Howships Lacunae on cortical bone (B) beneath abnormal periosteum. The medullary cavity (M) with its trabecular surfaces does not show resorptive activity. (Cat #7.)

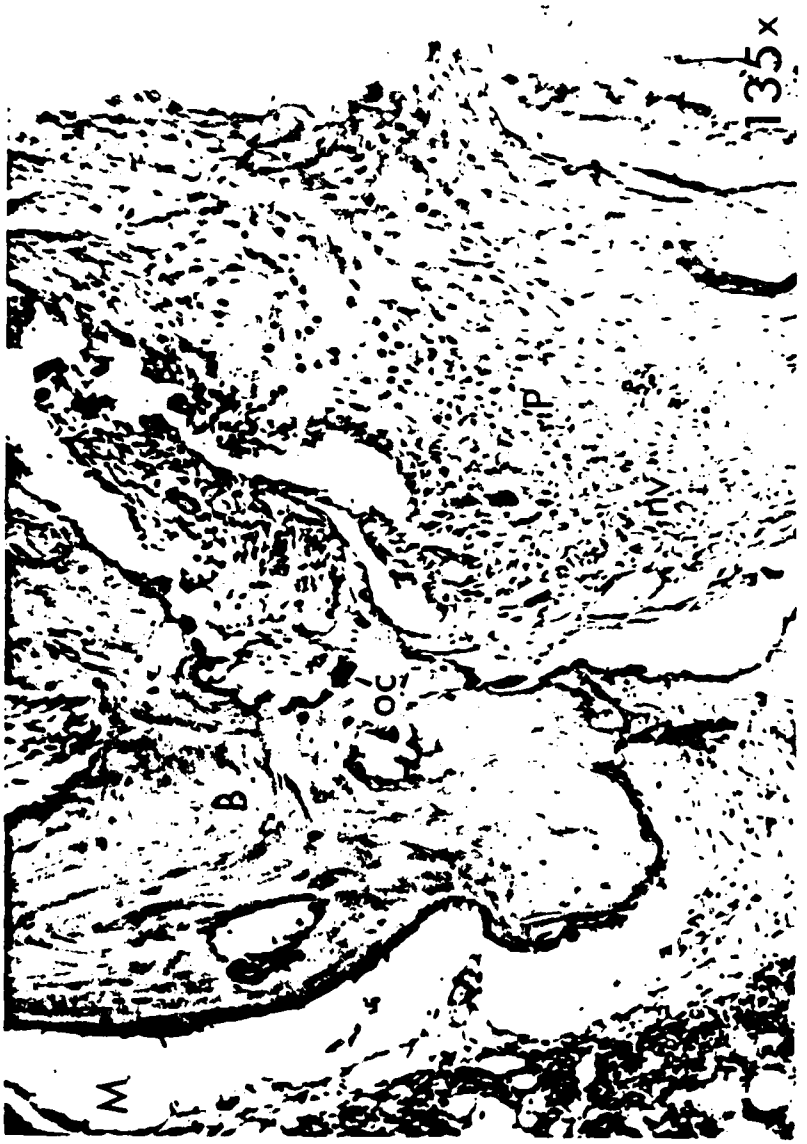
135×
P
nV
OC
B
M

Photo 2. Photomicrograph (537x) of a center of osteoclastic (OC) activity on the periosteal surface of cortical bone (B). (Cat #7.)

2

Photo 3. Photomicrograph (202x) of an area beneath joint cartilage (Ct) of a distal metatarsal, 1 month post-injury. Note fibrous infiltration (f) and vessels within the subchondral trabecular space. (Cat #7.)

3

in haversion systems, the two processes are nearly equal; on periosteal surfaces, formation normally exceeds resorption (8). In mature long bones, a gradual enlargement of the medullary cavity with slightly slower enlargement of the periosteal diameter causes a net loss of cortical bone and eventually a "physiological osteoporosis." Pathological forms of osteoporosis such as those following menopause, corticosteroid therapy, or long term immobilization appear similar to the physiologic osteoporosis in which endosteal bone loss is accelerated. Bone turnover actually may decrease in these diseases, but the resorption/formation ratio is enhanced. The effects are irreversible (9, 10). Posttraumatic and thyrotoxic osteoporosis differ from the above in that bone turnover is enhanced, all three envelopes are involved, and the changes are reversible.

Radiographic and biochemical studies of the animals examined histologically here support the diagnosis of true osteoporosis (Chapters 3 and 4). The most likely etiological factors involved are immobilization — in this case, disuse — and trauma. Most of the 18 animals studied favored the injured limb for several days post-injury, and four of them demonstrated pain on palpation of the foot for up to six weeks. Disuse atrophy of bone cannot be ruled out in the four cases. However, the remodeling patterns seen microscopically in this study are different from those seen with immobilization or disuse osteoporosis. Posttraumatic osteoporosis occurs only after injury; it can develop without immobilization of the part. Approximately eight weeks after a painful

injury, which may be a superficial one such as a burn or skin graft, radiographs show mottled or localized loss of density of cortical or cancelous bone. Small holes may be evident in radiographs of cortical bone. Histologically, there are active areas of remodeling on the cortex and trabeculae. Blood flow to the area is enhanced, and bone turnover rates are high. When the inflammatory response heals, the excessive bone loss stops; if the reaction is prolonged, the trabecular pattern may remain disturbed for years (9). Vascular changes are thought to be central to the pathogenesis of post-traumatic osteoporosis (11, 12).

Radiographic analysis of these animals demonstrates pathology similar to post-traumatic osteoporosis both in appearance of lesion and time of occurrence (Chapter 3). Local hyperemia of soft tissue was seen clinically and hyperemia of bone was indicated by enhanced ^{99}Tc uptake immediately post-injury. Increased vascularity was noted later on angiography (Chapter 3). The histological findings reported here showed evidence of enhanced bone turnover, especially in the periosteal envelope. Radiographic studies in man indicate that all but the localized subchondral lesions disappear by 4 years post-injury; thus the lesion is reversible (13). The results of this study support the hypothesis that post-frostbite osteoporosis falls in the category of post-traumatic osteoporosis.

SUMMARY

Bone samples from cold-injured and normal hind feet of 18 domestic cats were evaluated histologically. Subperiosteal osteoclastic resorption, the most common pathological finding, was seen as early as two weeks post-injury. Trabecular resorption, fibrous infiltration of trabecular spaces, and periosteal neovascularity were noted between 1 and 6 months post-injury. Histological findings showed that cold-induced bone resorption is specific, and distinct from that seen during immobilization.

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Chapter 6

STUDIES ON THE RESPONSE OF BONE TO ACUTE COLD EXPOSURE: CONCLUSIONS

The domestic cat is a suitable laboratory animal for the study of cold-injury. The cat, anesthetized with pentobarbital Na, responds with cold-induced vasodilatation and whole body thermoregulation when one hind foot is exposed to low temperature. The severity of the injury may be controlled by monitoring temperatures deep within the foot during freezing, thus cooling the foot to a selected temperature for a given period of time. The eventual frostbite injury appears clinically similar to frostbite in man, although the entire series of pathological events is condensed in time.

The radiographic lesions that develop after frostbite in the cat are similar in type and numerical distribution to those reported in man; the most common lesion noted is diffuse or localized radiolucency occurring 2-6 months post-injury. Periosteal abnormalities, early epiphyseal plate closure, and focal juxta-articular areas of decreased density are seen less frequently. "Rheumatoid-like punched out" juxta-articular lesions are less distinct and less likely to involve articular cartilage at six months post-injury than the analogous finding in man. These

subchondral lesions appear to be the last to develop in man; therefore, extending the time of radiographic observation in the injured cat might result in maturation of the lesion in that animal as well.

Active resorption of bone is evident microscopically before skeletal changes can be noted with standard radiographic techniques and may be found in animals that never demonstrate radiographic abnormalities. Periosteal proliferation and neovascularization, and subperiosteal resorption are seen as early as two weeks post-injury. Endosteal resorption and fibrous infiltration of trabecular spaces are observed on occasion. The most active remodeling of cortical bone appears to occur between 2 weeks and 2 months post-injury.

Clinical evaluation of the bone lesions while they are occurring is difficult. Whole body calcium balance is complex at best and cannot be evaluated easily. Serum calcium and phosphorus remain unchanged after injury while excretion of calcium and phosphorus in the urine is reduced 4-6 months post-injury; the latter finding suggests positive mineral balance and a formative phase in bone during that period. Studies of urine output of minerals would be more useful if calcium intake and fecal loss were measured. Serum alkaline phosphatase, thought to be an indicator of osteoblastic activity (i.e., either matrix formation or mineralization), is elevated from 2-4 months post-injury. The fundamental chemical determinations made here are helpful but only as they complement other data. There is no evidence that immunological processes are involved in cold-induced bone pathology in the cat. Fat-free dry

weight and percent ash results show that a true osteoporosis occurs even in bones that have suffered only very mild cold injury, i.e., the metatarsals.

The microangiographic technique used in the present study is a terminal procedure, but it provides a clear delineation of patent vasculature at the time of perfusion. Micro-extravasations, which indicate possible loss of vascular integrity, and tortuosity of vessels are seen as early as 2 days post-injury. An increased number of medullary vessels are evident in affected areas from 2 weeks through 6 months after injury. Contrast media forms feathery-edged pools within the medullary cavities of some injured bones. The finding is seen in conjunction with a radiographic diagnosis of endosteal scalloping or loss of cortical definition. The pooling may be the result of extravascular perfusion of the medullary cavity similar to that seen in aneurysmal bone cysts. Hyperemia of soft tissue is clearly evident immediately post-injury, and based on uptake of ^{99m}Tc, appears to be present in bone at 2 days, the time of the earliest post-injury bone scan. Histologically, neovascularity is evident in the periosteum and joint capsule of the frostbitten foot.

The nature and chronology of these findings support the hypothesis that a frostbite-induced increase in vascularity is an essential part of the pathogenesis of the bone lesions described above. The exact relationship is open to speculation.

HYPOTHESES

The ultimate mechanism of tissue injury from freezing is unknown. Cell injury or death may result from direct physical damage by ice crystals, from dehydration and concentration of solutes following freezing of intracellular water, or from ischemia secondary to a vascular lesion. Whatever the cause of cellular damage or death, the process results in the release of a multiplicity of chemical mediators, from tissue cells themselves and from blood cells and plasma factors drawn to or produced in the area as a part of the inflammatory response. When soft tissue is severely injured, it mummifies or becomes necrotic; the skeletal structures beneath it lose their blood supply and stop normal maintenance functions. The same thing occurs in bone when osteocytes are destroyed by the initial freeze injury. If revascularization occurs in damaged bone, severe lysis may result. When soft tissue and bone cells as well as blood vessels survive the initial cold assault, a part of the response may be hyperemia and the formation of new blood vessels. The hyperemia may result from the release of chemical mediators or damage to vasoconstrictor nerves that innervate the blood vessels of bones (1, 2, 3).

The extent to which neovascularization occurs in bone depends on the degree of injury; superficial injury may result only in periosteal reaction, while more severe injury that involves nutrient vessels or their venous drainage may enhance both the medullary and the periosteal blood supply (4). Bone is resorbed preferentially when blood flow or

vascularity is increased. The process appears to be a local one, as bone envelopes may be involved individually. The alkaline environment associated with increased blood flow is a factor that may link increased blood flow to acceleration of bone removal (4). The exact process by which "alkaline drift" enhances bone resorption has not been described, but it may work directly on hydroxyapatite crystals rather than through bone cells or parathyroid hormone activity. In tissue culture, a high pH depresses, rather than enhances, parathormone-induced bone resorption (5). A more likely possibility is that some factor produced locally by cells of infiltrating blood vessels or by cells that accompany the neovasculation stimulates osteoclastic differentiation through activation of the basic metabolic unit (6).

The osteoclast, which is the major cell responsible for normal and pathological resorption of bone, responds to parathyroid hormone, 1.25 dihydrocholecalciferol, and thyroxine (7, 8). Osteoclastic resorption is the major cause of bone loss in post-frostbite osteoporosis, but the local nature of the pathology precludes elevated parathyroid hormone levels as a means of activating osteoclasts. Alternative means of activation are suggested: (1) locally produced chemicals accompanying the post-injury reaction may activate osteoclasts directly, or (2) locally produced mediators may act synergistically to enhance the effectiveness of normal levels of parathyroid hormone. Osteoclast activating factor from leucocytes or lymphocytes, and PGE_2 from monocytes, fibrocytes, bone cells, or possibly vascular endothelium (9, 10) activates the

ruffled border resorbing apparatus of osteoclasts but does not stimulate initial development of osteoclasts (11). Prostaglandins are thought to act on receptor sites different from those that parathyroid hormone acts on and may or may not work through the adenylcyclase cAMP system as parathyroid hormone does (12). Some evidence indicates that PGE_2 can operate independently of parathyroid hormone (13), while other studies suggest that, at least in disuse osteoporosis, parathyroid hormone must be present, its activity being enhanced by local factors (14). Prostaglandins also may serve as regulators of the production of collagenase from macrophages (15). Collagenase acts in the resorption of bone matrix.

Both osteoclast activating factor and PGE_2 have been isolated from blood cells or cells of blood vessel walls. Evidence from previous related work suggests that these "local hormones" may play a part in cold-induced bone changes. The anatomical physiology of bone suggests that the elusive "bone softening factor" must be present in noninflammatory "hypervascularity." The soft metaphyseal trabecular bone is perfused from many capillary-like sinusoids supplied from large medullary nutrient vessels. Branches of the nutrient artery and post-capillary venules from the sinusoids pass centrifugally through the cortex, finally supplying the periosteum. The nature of bone metabolism within these three areas appears to have more than a casual relationship to its blood supply. The spongy character of bone within the normal metaphysis appears to be caused by resorption that exceeds formation. In the haversion systems of cortical bone, resorption and formation are nearly

equal, while in the periosteum, bone formation exceeds resorption (4). Increased production, increased effectiveness, or decreased destruction of prostaglandin or osteoclast activating factor in these more highly vascular areas of normal bone could explain the observations. The most reasonable explanation is that active products are synthesized in bone in response to a local stimulus related to the relative vascularity of the area in question.

The ultimate action of the activated osteoclast on bone is thought to involve the release of lysosomal enzymes from the area along the ruffled border of the cell in contact with the bone being resorbed (16). It is probable that several of the factors mentioned, and others besides, are involved in the restoration of bone following cold injury.

FURTHER STUDIES

Figure 1 depicts several sequences that are suggested as possible factors in the loss of bone following frostbite injury. Sites of potential experimental manipulation of the process of cold-induced bone destruction are indicated by numerals.

(1) Surgical manipulation of post-injury blood flow by ligation of the femoral vein would result in venous congestion, thus decreased pH and increased CO_2, without altering the normal inflammatory reaction. A gross differentiation of these two broad factors thought to be involved might be possible in this way.

(2) Chronic treatment with non-steroidal anti-inflammatory cyclo-

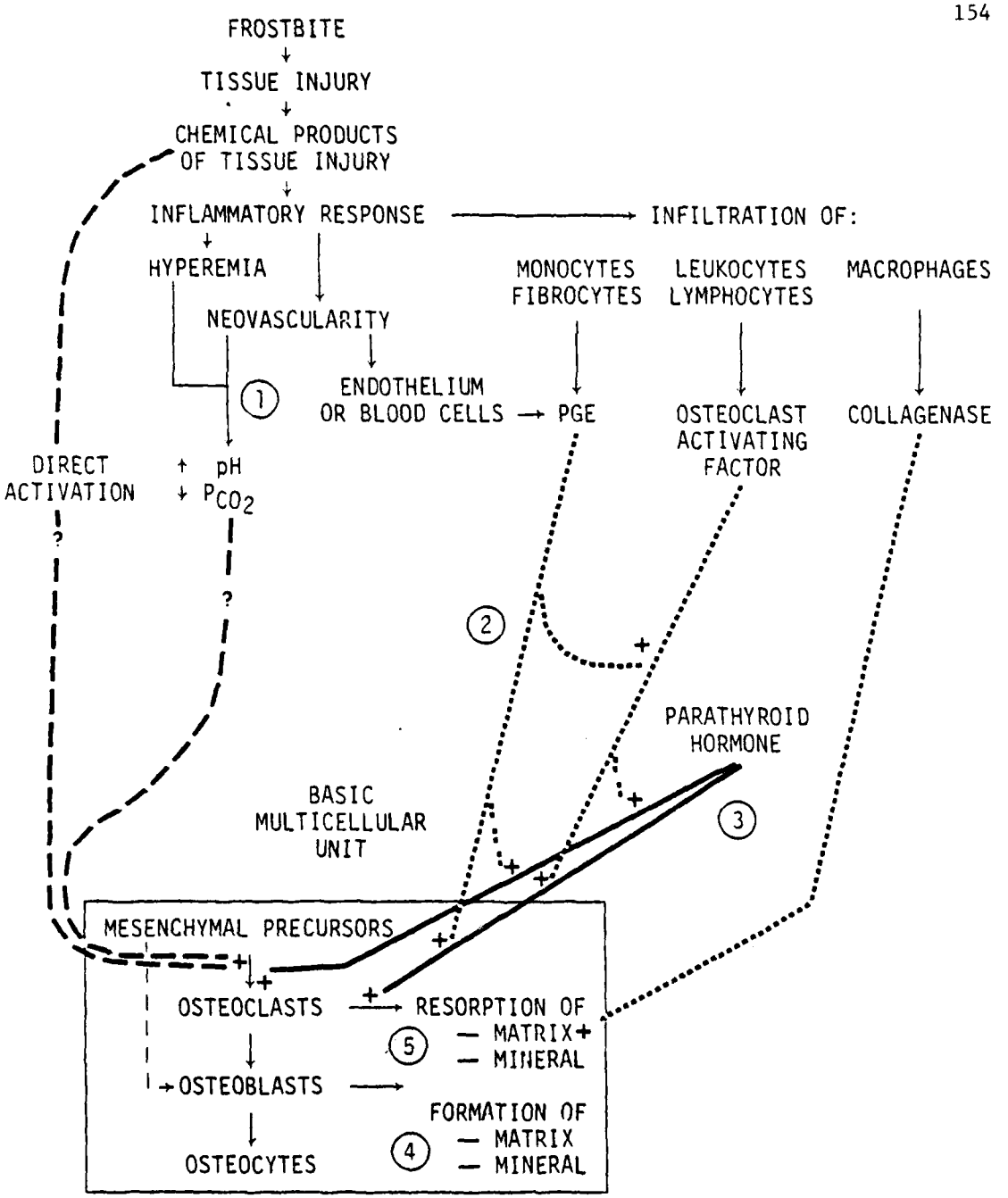

Figure 1. Flow chart depicting possible schemes in cold-induced bone lesions.

oxygenase inhibitors would block prostaglandin production and eliminate the proposed synergistic role of the prostaglandins with osteoclast activating factor and/or parathyroid hormone.

(3) Parathyroidectomy following cold injury might demonstrate the importance of parathyroid hormone in the subsequent resorption process.

(4) Tetracycline labeling of the calcification front at 30-60 day intervals would help to identify the proposed "formative phase" that may follow bone resorption.

(5) In the present study, serum and urine mineral levels were used as indicators of bone remodeling processes. Urine hydroxyproline, which reflects collagen breakdown, appears to be a better means of monitoring bone resorption.

Future studies should include microangiography before and after removal of periosteum from perfused bones. In the present work, the periosteum was removed before soft x-rays were made; this necessitated examination of periosteal neovascularity from histological sections alone.

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Appendix

COLLECTION OF URINE FROM CAGED LABORATORY CATS

During recent investigations, clean samples of urine and the measurement of total daily urine volume were required, but metabolism cages were not available. The purpose of this report is to describe a technique that was developed for collecting urine from caged laboratory cats.

MATERIALS AND METHODS

A total of 34 cats, 13 males and 21 females, were housed individually in fiberglass cages (60 x 54 x 79 cm) that were equipped with perforated, stainless-steel floors, elevated 8 cm above the fiberglass floor. All cats were allowed free access to dry cat foot and fresh water.

The method described here utilized a modified round rubber dish pan (Rubbermaid^{R} #2950-4, Rubbermaid Commercial Products, Overland Park, Kansas) (37 cm diameter x 13 cm deep). A small board (3 x 2 x 35 cm) was attached to the bottom of the pan, to provide a stable base and for drainage, by causing the pan to tilt approximately 6° from the horizon-

tal (Figure 1). Two small, flat-head wood screws were used to hold the board in place against the bottom of the pan, and a drop of clear epoxy was placed over the head of each screw to restore the smooth inner surface of the pan. The best location for the drain hole was established in advance by placing a marble in the tilted pan to indicate the lowest point in the outer circumference of the base of the pan. A hole 8 mm in diameter (number 5 cork borer) was punched at this position. The barrel of a 3 ml disposable syringe

was cut transversely at the 0.25 ml mark and pushed through the hole in the pan from the inside. To assure complete drainage of the pan, the finger grips on the plunger end of the syringe barrel were notched with a small hand-held electric grinder (Figure 1, insert). After a good fit was achieved, the syringe barrel was cemented in place with epoxy liquid adhesive. For better adhesion, the surfaces of both the pan and the syringe were roughened with fine sandpaper before the epoxy was applied. The modified pan was placed in the back corner of the cage with the drain hole away from the corner. The syringe barrel drain, which protruded through the floor, held a 100 ml disposable beaker in place under the elevated cage floor.

RESULTS AND DISCUSSION

This technique has been used for more than 200 cat-days with a collection success rate of more than 82%. Failures included loose stools contaminating the urine (3%), stool blocking the drain hole (3%),

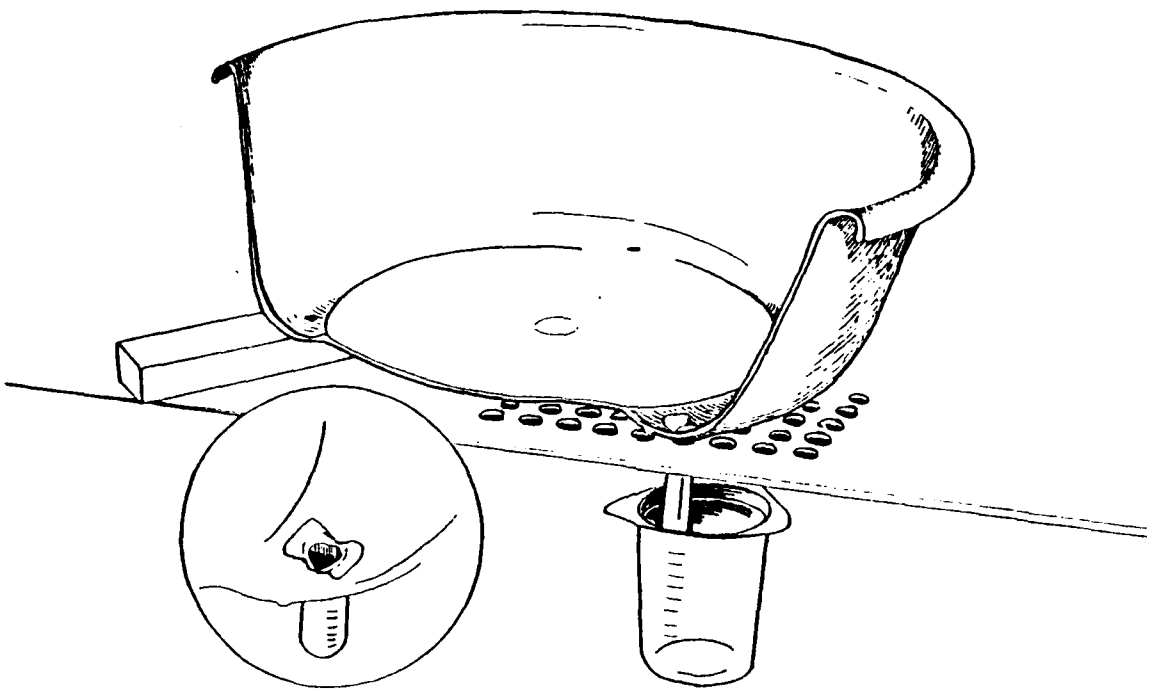


Figure 1. Pan from which one side has been removed to show inner details. Inset illustrates modified syringe barrel drain.

the pan moved from over the beaker (3%), or the cat urinating outside of the pan or not urinating at all (9%). Rectangular, stainless-steel pans filled with wood-shavings were used for 4-5 days before the first collection was attempted. Collection of urine was attempted from 17 cats, 5 days after their arrival in our laboratory. Only three of the 17 cats did not urinate in the collection pan on the first day; all cats used the new pan on the second day. A second group of 17 cats was housed for 4 days before the first attempt to collect samples of urine. Six cats of this group used the pan on the first day, five more used it on the second day, and all 17 used it by the fourth day. After using the collection pan one time, most cats continued to use it whenever it was placed in their cages.

On occasion, hyperactive animals tipped the pan or moved the drain from one hole in the perforated floor to another, causing loss of the urine sample. This problem was avoided by proper placement of the pan with the high side positioned into the back corner of the cage. On several occasions, rubber bands were attached from the rim of the pan to the cage floor to provide extra stability (Figure 2).

The urine specimens collected by this method normally are free of contamination and suitable for chemical analysis. Because cats use the pan for defecation as well as urination, a soft stool, or a normal stool deposited over the drain, may contaminate or cause the loss of urine. Fortunately, cats normally urinate immediately before they defecate, and then only once in a 24-hour period; therefore, contamination

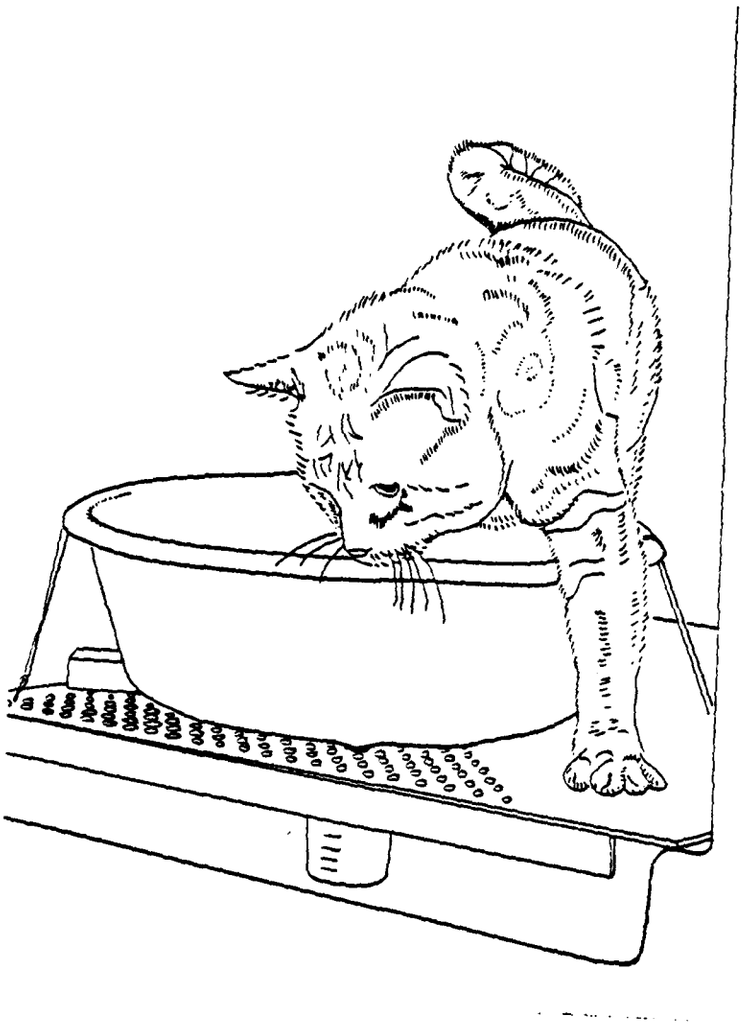
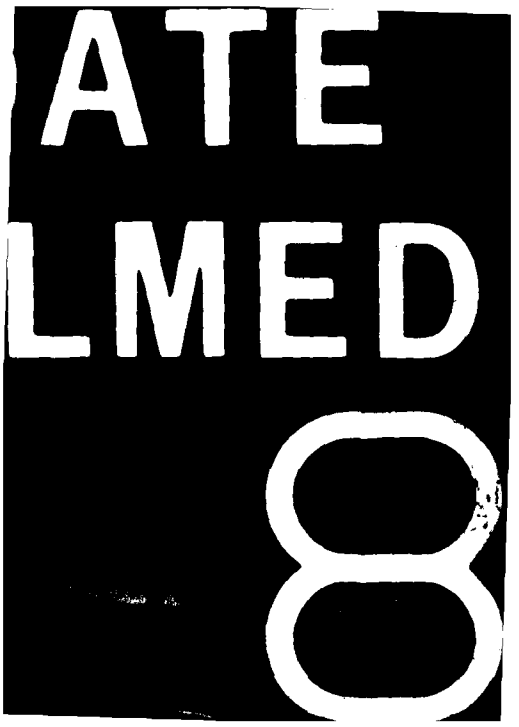


Figure 2. Pan in place on cage floor. Note rubber bands which add stability.

of the specimen by feces is uncommon. A normal, or even slightly soft, stool remains where it is deposited on the gently sloping bottom of the pan; thus, it does not contaminate the urine in the beaker below. The volume of urine collected is essentially 100% of the urine that is voided; thus, the technique is useful for volume studies.

SUMMARY

Twenty-four hour urine specimens were collected from caged domestic cats using modified rubber dish-pans. This simple method provided both an uncontaminated specimen and total daily urine output, and required no training of the animals.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